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*Publication date:*  
2010

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Storgaard, M. (2010). *Design, Synthesis and Biological Activity of Novel Reversible Peptidyl FVIIa Inhibitors Rh-Catalyzed Enantioselective Synthesis of Diaryl Amines*. Technical University of Denmark.

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# Design, Synthesis and Biological Activity of Novel Reversible Peptidyl FVIIa Inhibitors

## Rh-Catalyzed Enantioselective Synthesis of Diaryl Amines



Morten Storgaard

*PhD-Thesis*

January 2010

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**Morten Storgaard**  
*PhD-Thesis*

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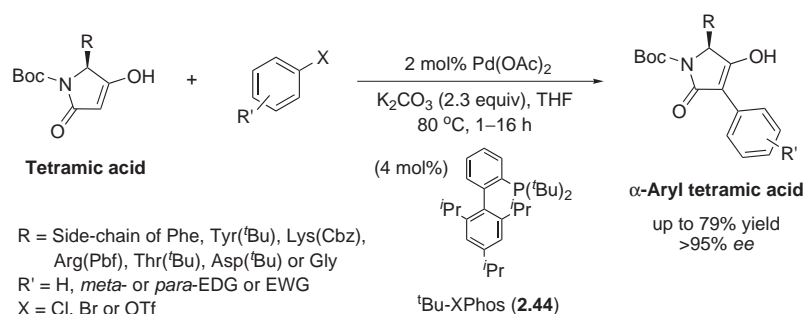
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January 2010

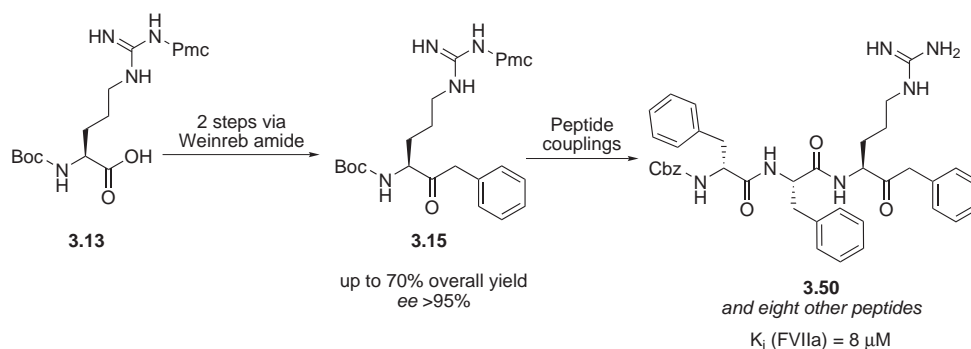
## Abstract in English

This thesis describes two different projects. The first project deals with the design, synthesis and biological activity of novel reversible peptidyl FVIIa inhibitors (Chapter 1–3). FVIIa was launched as NovoSeven<sup>®</sup> over a decade ago by Novo Nordisk A/S for the treatment of hemophilia A and B complicated by antibodies. FVIIa is a serine protease and hence liquid formulations are not stable due to autoproteolysis. A reversible inhibitor would stabilize FVIIa making a liquid formulation possible, representing an important follow-up product for Novo Nordisk A/S. Peptidyl benzyl ketones was chosen as a new class of potential inhibitors, whose sequence was rationally selected from a previously reported FVIIa-TF specificity profile.

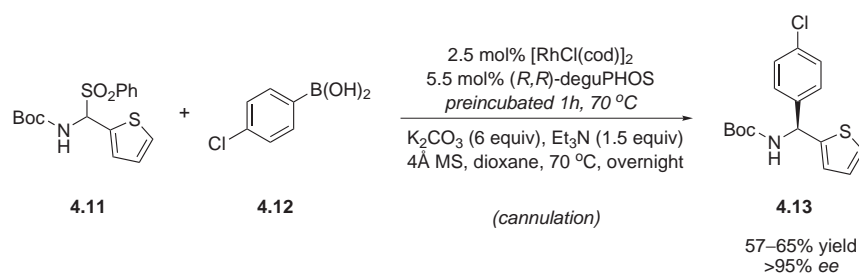
Since arginine was found to be the most active P1-amino acid, a mild and efficient synthesis of the corresponding arginyl benzyl ketone building block was sought. Two strategies were proposed, the one involving a tetramic acid key intermediate being the most straightforward. For introduction of the benzyl functionality, a palladium-catalyzed  $\alpha$ -arylation was developed. This transformation occurs under mild conditions showing high functional group tolerance. Unfortunately, these  $\alpha$ -aryl tetramic acids were too unreactive and ring opening toward the synthesis of the building block did not succeed. However,  $\alpha$ -aryl tetramic acids are interesting compounds due to their potential biological activity.



The building block **3.15** (P1) was instead synthesized via a Weinreb amide and a small library of peptides were prepared by solution-phase Boc/Bn-synthesis. Different P3-P2 sequences (tyrosine, threonine, phenylalanine, leucine) and *N*-terminals (P4; H-, Ac-, BnSO<sub>2</sub>- and Cbz-) were examined. Unfortunately, *O*-debenzylation was found to be very difficult which restricted the number of peptides containing tyrosine and threonine. Cbz-D-Phe-Phe-Arg-bk (**3.50**) was identified as the most potent FVIIa inhibitor with a  $K_i = 8 \mu\text{M}$  ( $\text{IC}_{50} = 16 \mu\text{M}$ ) and with a 35- and 28-fold selectivity against thrombin and FXa, respectively. A SAR revealed that a bulky aromatic Cbz-terminal was crucial for potency.



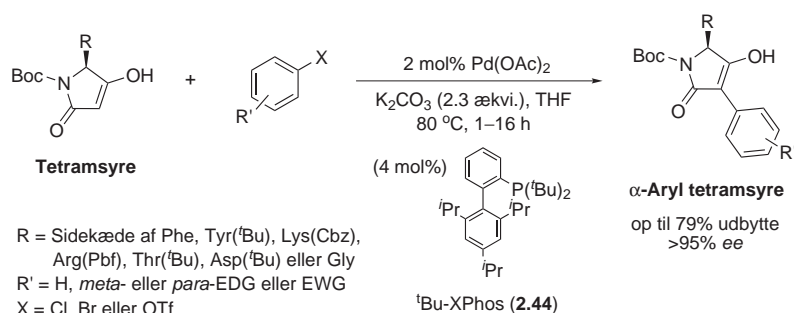
The second project deals with the rhodium-catalyzed enantioselective synthesis of diaryl amines, which is an important class of compounds (Chapter 4). For example it is found in the third generation anti-histaminic agent levocetirizine. Development of efficient synthetic routes is therefore of considerably interest. The rhodium-catalyzed enantioselective synthesis employing  $\alpha$ -carbamoyl sulfones and arylboronic acids was therefore investigated using the chiral ligand (*R,R*)-deguPHOS.  $\text{Rh}(\text{acac})(\text{coe})_2$  was originally utilized requiring the use of a glovebox, but through catalyst screening  $[\text{RhCl}(\text{cod})]_2$  was found to be equally efficient. Contrary to  $\text{Rh}(\text{acac})(\text{coe})_2$ , this new catalyst is air-stable, commercially available and inexpensive.  $[\text{RhCl}(\text{cod})]_2$  and (*R,R*)-deguPHOS was preincubated prior to use to secure excellent enantioselectivity. A cannulation technique was implemented for application outside the glovebox. A low content of boroxine in the arylboronic acid batch was found to be crucial for a satisfactory outcome. The highly functionalized  $\alpha$ -diaryl methylamine **4.13** was synthesized in good yield and excellent enantioselectivity in gram-scale. The absolute configuration was determined by X-ray crystallography to be the (*S*)-enantiomer.



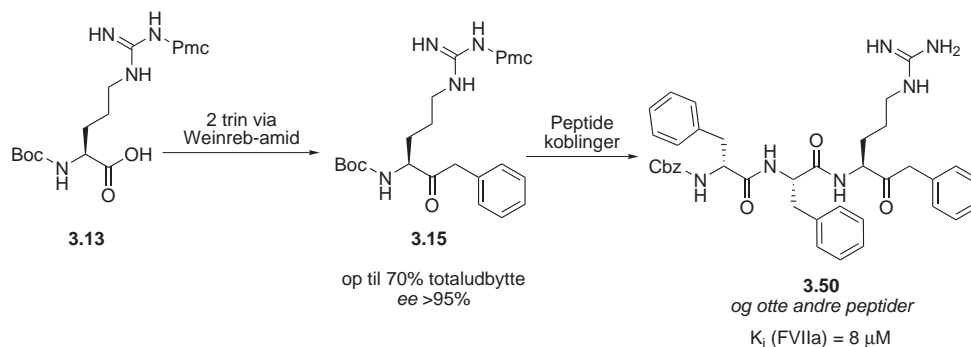
## Resumé på dansk

Denne afhandling omhandler to forskellige projekter. Det første projekt omhandler design, syntese og biologisk aktivitet af nye, reversible peptid-baserede FVIIa-inhibitorer (Kapitel 1–3). FVIIa blev lanceret som NovoSeven<sup>®</sup> for over 10 år siden af Novo Nordisk A/S for behandling af hæmofili A og B kompliceret af antistoffer. FVIIa er en serine-protease og flydende formuleringer er derfor ikke stabile på grund af autoproteolyse. En reversibel inhibitor der stabiliserer FVIIa ville kunne gøre en flydende formulering mulig, og dermed udgøre en vigtig produktudvidelse for Novo Nordisk A/S. Peptid-baserede benzylketoner blev valgt som en ny klasse af potentielle inhibitorer hvis sekvens rationelt blev udvalgt på baggrund af en tidligere publiceret FVIIa-TF specificitetsprofil.

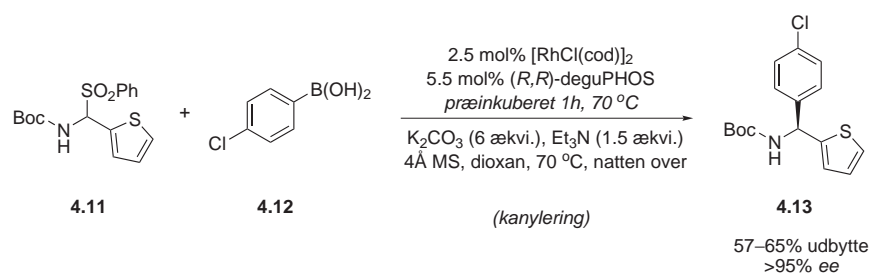
Da arginin blev fundet til at være den mest aktive P1-aminosyre, var det ønsket at udvikle en mild og effektiv syntese af den tilsvarende arginyl benzylketon-byggeblok. To strategier blev foreslået, men den strategi der involverer en tetramsyre som nøgleintermediat var den mest enkle. En palladium-katalyseret  $\alpha$ -arylering blev udviklet for introduktion af benzyl-funktionaliteten. Denne reaktion forløber under milde betingelser og udviser høj tolerance overfor funktionelle grupper. Desværre er  $\alpha$ -aryl tetramsyre ureaktive og ringåbning for syntese af byggeblokken var derfor ikke succesfuld. Imidlertid udgør disse stoffer en interessant gruppe på grund af deres potentielle biologiske aktivitet.



Byggeblokken **3.15** (P1) blev i stedet syntetiseret via et Weinreb-amid og et lille bibliotek af peptider blev fremstillet ved solution-phase Boc/Bn-syntese. Forskellige P3-P2 sekvenser (tyrosin, threonin, phenylalanin, leucin) og *N*-terminaler (P4; H-, Ac-, BnSO<sub>2</sub>- and Cbz-) blev undersøgt. Desværre viste det sig, at *O*-debenzyleringen var svær at udføre hvilket gav en begrænsning på antallet af peptider indeholdende tyrosin og threonin. Cbz-D-Phe-Phe-Arg-bk (**3.50**) blev fundet til at være den mest potente FVIIa inhibitor med  $K_i = 8 \mu\text{M}$  og med en selektivitet på 35- og 28-gange overfor hhv. thrombin og FXa. En struktur-aktivitetsanalyse viste, at Cbz-terminalen og D-aminosyren var afgørende for at opnå lav-mikromolær aktivitet. Trods moderat aktivitet og selektivitet samt lav opløselighed i vandige medier, udgør denne inhibitor en rimelig kandidat for den videre udvikling af flydende formuleringer af FVIIa.



Det andet projekt omhandler den rhodium-katalyserede enantioselektive syntese af di-arylaminer, som er en vigtig stofklasse (Kapitel 4). Foreksempel findes den i tredje-generations antihistaminet levocetirizin. Udvikling af effektive synteseveje er derfor af stor interesse. Den rhodium-katalyserede enantioselektive syntese med den chirale ligand (*R,R*)-deguPHOS og  $\alpha$ -carbamoysulfoner og arylborsyrer som udgangsstoffer blev undersøgt. Rh(acac)(coe)<sub>2</sub> var den oprindelige katalysator, som tillige krævede anvendelsen af en handskebox. Gennem screening blev [RhCl(cod)]<sub>2</sub> fundet til at en lige så effektiv katalysator. Den er tilmed luftstabil, kommerciel tilgængelig og billig i modsætning til Rh(acac)(coe)<sub>2</sub>. [RhCl(cod)]<sub>2</sub> og (*R,R*)-deguPHOS blev præinkuberet før brug for at opnå en høj enantioselektivitet. En kanyleringsteknik blev implementeret for at kunne udføre reaktionen udenfor handskeboxen. Et lavt indhold af boroxin i arylborsyren blev fundet at være afgørende for et tilfredsstillende resultat. Den højtfunctionaliseret  $\alpha$ -diarylmethylamin **4.13** blev syntetiseret i gram-skala i godt udbytte og høj enantioselektivitet. Den absolutte konfiguration blev bestemt ved røntgen-kristallografi til at være (*S*)-enantiomeren.







## Preface

This PhD thesis consists of six chapters covering two independent projects. The first project is 'Design, Synthesis and Biological Activity of Novel Reversible Peptidyl FVIIa Inhibitors' (Chapter 1–3) and the second project is 'Rhodium-Catalyzed Enantioselective Synthesis of Diaryl Methyl Amines' (Chapter 4). An overall list of references (Chapter 5) and list of related publications (Chapter 6) are also included. References are numbered consecutively throughout the entire thesis, whereas compounds are numbered consecutively according to which chapter they appear in: e.g. **3.2** is compound no. 2 in Chapter 3. Peptide nomenclature is used whenever appropriate. Chymotrypsin numbering of amino acid residues in FVIIa is used throughout the thesis. Supporting information, such as NMR spectra and HPLC chromatograms are available electronically upon request, if not included with this hardcopy. The first project (January 2007 to May 2008 and December 2008 to January 2010) was carried out at Novo Nordisk A/S, Måløv in the Department of Biopharm Chemistry under the supervision of Dr. Bernd Peschke (Novo Nordisk A/S) and Professor David Tanner from The Technical University of Denmark (DTU), Department of Chemistry. The second project (June to November 2008) was carried out at University of California at Berkeley, College of Chemistry under the supervision of Professor Jonathan ('Jon') A. Ellman.

First of all, I would like to address a thanks to my two supervisors, Bernd and David. They have both provided me with great inspiration throughout the years. Bernd was always ready to help me during my stay at Novo Nordisk and providing me with feedback, whenever I needed it. With his huge knowledge of industrial drug discovery, he was a valuable mentor. On the other hand, David was the best supervisor in organic chemistry you could ask for. He gave me many good advices and he taught me a lot of organic chemistry. Bernd and David are also thanked for proofreading this thesis.

Not to forget, I would like to thank my two former supervisors at Novo Nordisk; Dr. Janne E. Tønder and Dr. Florencio ('Flo') Zaragoza Dörwald. Janne was the one who made it possible for me to do my PhD studies at Novo Nordisk, but unfortunately she had to leave the company after just one month due to the sudden discontinuation of the small molecule research. However, I am grateful to her for the effort that she put in the PhD application and for her everlasting enthusiasm and friendly spirit. Flo was my supervisor for approximately seven months thereafter, when he decided to leave Denmark to take up a job at Lonza in Switzerland. He was the one who formulated the final project regarding design and synthesis of FVIIa inhibitors. Despite our short time of collaboration, he taught me many valuable experimental skills and I appreciate his profound knowledge of chemistry.

I would also like to thank all employees in Biopharm Chemistry and Diabetes ProPep Chemistry at Novo Nordisk A/S, but in particular Alice Ravn, Helle Selvig, Johnny Madsen and Lars Linderøth who were my lab-mates throughout the years. We had a great time in the C9.2.08-lab, which I will definitely miss. Moreover, I would like to thank Henrik Stephensen for tuning the HRMS, Claus Bruun for shimming the NMR instrument, Paw Block for making sure that all the HPLC/UPLC's are running perfectly, Sonja Bak for providing me with solvents and letting me borrow a lot of her lab-equipment, Johnny for helping me with the preparative HPLC and Helle with the lyophilization. Rie Kristine Schjeltved, Carsten Behrens and Henning Stennicke are all thanked for teaching me how to perform the competitive binding assays. Berit Lassen is thanked for providing me with hFXa.

Novo Nordisk and Corporate Research Affairs (CORA) are thanked for financial support. Berit Albrechtsen, Marianne Søndergaard and Lis Vejle Pedersen are thanked for helping me out with all the administrative work. Moreover, I would like to thank Oticon Fonden, Augustinus Fonden and Ingeniør Alexandre Haynman and Hustru Nina Haynmans Fond for additional financial support during my stay in the USA.

At the Department of Chemistry (DTU) I would like to thank all employees in building 201. Many of you have been there since my early beginning in 2001 as a first-year chemistry student, and it was always great to be back, although my visits were usually quite short. Dr. Masood Hosseini is thanked for many fun hours and great scientific discussions.

Last but not least, I would like to address a thanks to Jon for letting me do research in his group. He was a very enthusiastic supervisor and he was always ready to provide me with new and interesting inputs to the project. In addition, I would like to thank the entire Ellman group for being very friendly and helpful. Especially, I would like to thank Melissa Leyva, Denise Colby, MaryAnn Robak and Tyler Baguley for making the 908 Latimer Hall-lab a good place to work. Denise is moreover thanked for helping me with the glovebox and MaryAnn for preparing crystals for X-ray crystallography. Besides of the fun time I had with the people from the Ellman group, my 60 house-mates in the Hillegass-Parker coop definately also made my stay in the USA funny and memorable, and in particular they made it more vegetarian...

**Morten Storgaard, M.Sc.Eng.**

January 2010

## Abbreviations

|                                 |  |
|---------------------------------|--|
| $[\alpha]_D^{20}$               | Optical rotation (sodium D line at 20 °C)  |
| <b>A</b>                        | Alanine  |
| <b>Ac</b>                       | Acetyl   |
| <b>ACC</b>                      | 7-Amino-4-carbamoylmethylcoumarin  |
| <b>Ac<sub>2</sub>O</b>          | Acetic acid anhydride  |
| <b>AcOH</b>                     | Acetic acid  |
| <b>acac</b>                     | Acetylacetonate  |
| <b>Ala</b>                      | Alanine  |
| <b>Arg</b>                      | Arginine   |
| <b>Asn</b>                      | Asparagine   |
| <b>Asn*</b>                     | Glycosylated asparagine  |
| <b>Asp</b>                      | Aspartic acid  |
| <b>AT</b>                       | Antithrombin   |
| <b>ATR</b>                      | Attenuated total reflection  |
| <b>BAL</b>                      | Backbone amide linker  |
| <b>9-BBN</b>                    | 9-Borabicyclo[3.3.1]nonane   |
| <b>BINAP</b>                    | 2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl  |
| <b>Boc</b>                      | <i>tert</i> -Butoxycarbonyl  |
| <b>Boc<sub>2</sub>O</b>         | Di- <i>tert</i> -butyl dicarbonate   |
| <b>bod*</b>                     | Bicyclo[2.2.2]octadiene  |
| <b>BOP</b>                      | Benzotriazolyl- <i>N</i> -oxytrisdimethylaminophosphonium PF <sub>6</sub> <sup>-</sup> |
| <b>bk</b>                       | Benzyl ketone  |
| <b>Bn</b>                       | Benzyl   |
| <b>BnBr</b>                     | Benzylbromide  |
| <b>BnOH</b>                     | Benzyl alcohol or phenylmethanol   |
| <b>BnSO<sub>2</sub>Cl</b>       | Phenylmethanesulfonyl chloride   |
| <b>Bt</b>                       | Benzotriazole  |
| <b>Bzl</b>                      | Benzyl   |
| <b>calcd.</b>                   | Calculated   |
| <b>CaR</b>                      | Calcium-sensing receptor   |
| <b>Cbz</b>                      | Benzyloxycarbonyl  |
| <b>CDI</b>                      | Carbonylimidazole  |
| <b>Cha</b>                      | Cyclohexylalanine  |
| <b><sup>c</sup>Hex-JohnPhos</b> | 2-(Dicyclohexylphosphino)biphenyl  |
| <b>CLND</b>                     | Chemiluminescent nitrogen detection  |
| <b>cmk</b>                      | Chloromethyl ketone  |
| <b>coe</b>                      | Cyclooctaene   |
| <b>cod</b>                      | Cyclooctadiene   |
| <b>conc.</b>                    | Concentrated   |

|                        |   |
|------------------------|---|
| <b>18-Crown-6</b>      | 1,4,7,10,13,16-Hexaoxacyclooctadecane                           |
| <b>D</b>               | Aspartic acid   |
| <b>DavePhos</b>        | 2-Dicyclohexylphosphino-2'-( <i>N,N</i> -dimethylamino)biphenyl |
| <b>dba</b>             | Dibenzylideneacetone  |
| <b>DBU</b>             | 1,8-Diazabicyclo[5.4.0]undec-7-ene                              |
| <b>DCC</b>             | <i>N,N'</i> -Dicyclohexylcarbodiimide                           |
| <b>DCM</b>             | Dichloromethane   |
| <b>de</b>              | Diastereomeric excess   |
| <b>deguPHOS</b>        | (1-Benzyl-3,4-bis(diphenylphosphino)pyrrolidine                 |
| <b>decomp.</b>         | Decomposed  |
| <b>DHUA</b>            | 10,11-Dihydroxy-undecanoic acid                                 |
| <b>DIBAL-H</b>         | Diisobutylaluminium hydride                                     |
| <b>DIC</b>             | <i>N,N'</i> -Diisopropylcarbodiimide                            |
| <b>DIPEA</b>           | Diisopropylethylamine   |
| <b>DMAP</b>            | <i>N,N</i> -Dimethyl-4-aminopyridine                            |
| <b>DMF</b>             | Dimethyl formamide  |
| <b>DMSO</b>            | Dimethylsulphoxide  |
| <b>DNA</b>             | Deoxyribonucleic acid   |
| <b>dppbenz</b>         | 1,2-Bis(diphenylphosphino)benzene                               |
| <b>DPPF</b>            | 1,1'-Bis(diphenylphosphino)ferrocene                            |
| <b>dr</b>              | Diastereomeric ratio  |
| <b>DTU</b>             | The Technical University of Denmark                             |
| <b>E</b>               | Glutamic acid   |
| <b>EDC</b>             | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride     |
| <b>EDG</b>             | Electron-donating group   |
| <b>ee</b>              | Enantiomeric excess   |
| <b>EGF</b>             | Epidermal growth factor-like domain                             |
| <b>elem. anal.</b>     | Elemental analysis  |
| <b>equiv</b>           | Equivalent  |
| <b>ESI</b>             | Electrospray ionization   |
| <b>Et</b>              | Ethyl   |
| <b>Et<sub>3</sub>N</b> | Triethyl amine  |
| <b>Et<sub>2</sub>O</b> | Diethyl ether   |
| <b>EtOAc</b>           | Ethyl acetate   |
| <b>EtOH</b>            | Ethanol   |
| <b>EtOTs</b>           | Ethyl tosylate or ethyl 4-toluene sulphonate                    |
| <b>EWG</b>             | Electron-withdrawing group                                      |
| <b>F</b>               | Phenylalanine   |
| <b>FDA</b>             | Food and Drug Administration (USA)                              |
| <b>Fmoc</b>            | 9-Fluorenylmethoxycarbonyl                                      |
| <b>FT-IR</b>           | Fourier transform infrared spectroscopy                         |

|                         |  |
|-------------------------|--|
| <b>FVa</b>              | Activated factor V   |
| <b>FVII</b>             | Factor VII   |
| <b>FVIIa</b>            | Activated factor VII   |
| <b>FIX</b>              | Factor IX  |
| <b>FIXa</b>             | Activated factor IX  |
| <b>FX</b>               | Factor X   |
| <b>FXa</b>              | Activated factor X   |
| <b>FX act.</b>          | Degree of FX activation  |
| <b>FXIa</b>             | Activated factor XI  |
| <b>G</b>                | Glycine  |
| <b>Gla</b>              | Glutamic acid-rich domain  |
| <b>Gln</b>              | Glutamine  |
| <b>Glu</b>              | Glutamic acid  |
| <b>Gly</b>              | Glycine  |
| <b>GlyGly</b>           | Glutylglycine (diglycine)  |
| <b>H</b>                | Histidine  |
| <b>h</b>                | Hour   |
| <b>H<sub>1</sub></b>    | Histamine 1 (receptor)   |
| <b>HATU</b>             | <i>O</i> -(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium PF <sub>6</sub> <sup>-</sup>                   |
| <b>HBTU</b>             | <i>O</i> -(Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium PF <sub>6</sub> <sup>-</sup>                        |
| <b>HEPES</b>            | (4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid  |
| <b>hFXa</b>             | Human activated factor X   |
| <b>His</b>              | Histidine  |
| <b>HIV</b>              | Human immunodeficiency virus   |
| <b>HMPA</b>             | Hexamethylphosphoramide  |
| <b>HOAt</b>             | 7-Aza-1-hydroxybenzotriazole   |
| <b>HOBt</b>             | 1-Hydroxy-1 <i>H</i> -benzotriazole  |
| <b>HONb</b>             | <i>N</i> -Hydroxy-5-norbornene-2,3-dicarboxylimide   |
| <b>HPLC</b>             | High performance liquid chromatography   |
| <b>HRMS</b>             | High resolution mass spectroscopy  |
| <b>HSTU</b>             | <i>O</i> -( <i>N</i> -Succimidyl)- <i>N,N,N',N'</i> -bis(tetramethylene)uronium PF <sub>6</sub> <sup>-</sup> |
| <b>HTS</b>              | High throughput screening  |
| <b>I</b>                | Isoleucine   |
| <b>IBCF</b>             | Isobutyl chloroformate   |
| <b>IC<sub>50</sub></b>  | Half maximal inhibitory concentration  |
| <b>Ile</b>              | Isoleucine   |
| <b>IPCF</b>             | Isopropenyl chloroformate  |
| <b><sup>i</sup>Pr</b>   | Isopropyl  |
| <b><sup>i</sup>PrOH</b> | Isopropyl alcohol or 2-propanol  |
| <b>IR</b>               | Infrared spectrometry  |
| <b>J</b>                | <i>J</i> -coupling or indirect dipole dipole coupling  |

|                        |  |
|------------------------|--|
| <b>JohnPhos</b>        | 2-(Di- <i>tert</i> -butylphosphino)biphenyl                          |
| <b>K</b>               | Lysine   |
| <b>KHMDS</b>           | Potassium bis(trimethylsilyl)amide                                   |
| <b>L</b>               | Ligand or leucine  |
| <b>LC-MS</b>           | Liquid chromatography mass spectroscopy                              |
| <b>LDA</b>             | Lithium diisopropylamide   |
| <b>Leu</b>             | Leucine  |
| <b>LMWH</b>            | Low molecular weight heparins  |
| <b>L-Selectride</b>    | Lithium tri- <i>sec</i> -butylborohydride                            |
| <b>Lys</b>             | Lysine   |
| <b>K<sub>i</sub></b>   | Binding affinity   |
| <b>m-CPBA</b>          | <i>meta</i> -Chloroperoxybenzoic acid                                |
| <b>Me</b>              | Methyl   |
| <b>MeCN</b>            | Acetonitrile   |
| <b>MeOH</b>            | Methanol   |
| <b>MePhos</b>          | 2-Dicyclohexylphosphino-2'-methylbiphenyl                            |
| <b>Me<sub>2</sub>S</b> | Dimethyl sulfide   |
| <b>MS</b>              | Molecular sieves or mass spectrometry                                |
| <b>MSD</b>             | Mass storage device  |
| <b>Mtr</b>             | (4-Methoxy-2,3,6-trimethylphenyl)sulfonyl                            |
| <b>M<sub>w</sub></b>   | Molecular weight   |
| <b>m/z</b>             | Mass-to-charge ratio   |
| <b>N</b>               | Asparagine   |
| <b>Na(acac)</b>        | Sodium acetylacetonate   |
| <b>NaHMDS</b>          | Sodium bis(trimethylsilyl)amide                                      |
| <b>NaOEt</b>           | Sodium ethoxide  |
| <b>NaOtBu</b>          | Sodium <i>tert</i> -butoxide   |
| <b>Nbb</b>             | 2-Nitrobenzyl bromide  |
| <b>NMM</b>             | <i>N</i> -Methylmorpholine   |
| <b>NMR</b>             | Nuclear Magnetic Resonance   |
| <b>Ns</b>              | Nosyl (4-nitrobenzenesulfonyl)                                       |
| <b>OD</b>              | Optical density or absorbance  |
| <b>OEG</b>             | Oligo(ethoxy)ethylene glycol (oligo(8-amino 3,6-dioxaoctanoic acid)) |
| <b>Orn</b>             | Ornithine  |
| <b>OSu</b>             | <i>O</i> -Succinimidyl ester   |
| <b>P</b>               | Proline  |
| <b>P*</b>              | Chiral phosphine ligand  |
| <b>PAF</b>             | Platelet activating factor   |
| <b>PAL</b>             | Peptide amide linker   |
| <b>Pbf</b>             | 2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl                    |
| <b>PEG</b>             | Polyethylene glycol  |

|                            |  |
|----------------------------|--|
| <b>Pfp</b>                 | Pentafluorophenyl  |
| <b>Pg</b>                  | Protection group   |
| <b>Ph</b>                  | Phenyl   |
| <b>PhDavePhos</b>          | 2-Diphenylphosphino-2'-( <i>N,N</i> -dimethylamino)biphenyl                                |
| <b>Phe</b>                 | Phenylalanine  |
| <b>PhSH</b>                | Thiophenol   |
| <b>Phth</b>                | Phthaloyl  |
| <b>Pip</b>                 | Pipecolic acid or piperidine-2-carboxylic acid   |
| <b>PMA</b>                 | Phosphomolybdic acid   |
| <b>Pmc</b>                 | 2,2,5,7,8-Pentamethylchroman-6-sulfonyl  |
| <b>Pn</b>                  | Amino acid residue   |
| <b>pNA</b>                 | <i>para</i> -Nitro anilide or <i>para</i> -Nitro aniline                                   |
| <b>PPh<sub>3</sub></b>     | Triphenylphosphine   |
| <b>PPTS</b>                | Pyridinium <i>p</i> -toluenesulfonate  |
| <b>Pro</b>                 | Proline  |
| <b>PS</b>                  | Polystyrene  |
| <b>psi</b>                 | Pound per square inch  |
| <b>PS-SCL</b>              | Positional scanning, synthetic combinatorial libraries                                     |
| <b>PyBOP</b>               | Benzotriazol-1-yl-oxytri(pyrrolidino)phosphonium PF <sub>6</sub> <sup>-</sup>              |
| <b>Q</b>                   | Glutamine  |
| <b>R</b>                   | Arginine   |
| <b>rac</b>                 | Racemic  |
| <b>R<sub>f</sub></b>       | Retention factor   |
| <b>rFVIIa</b>              | Recombinant activated factor VII   |
| <b>rFVIII</b>              | Recombinant factor VIII  |
| <b>rFIX</b>                | Recombinant factor IX  |
| <b>rFXIII</b>              | Recombinant factor XIII  |
| <b>rt</b>                  | Room temperature   |
| <b>S</b>                   | Serine   |
| <b>Sn</b>                  | Binding site/pocket  |
| <b>SAR</b>                 | Structure activity relationship  |
| <b>Sat.</b>                | Saturated  |
| <b>Ser</b>                 | Serine   |
| <b>(S)-ShiP</b>            | Phenyl-[( <i>S</i> )-1,1-spirobiindane-7,7-diyl]-phosphite                                 |
| <b>S<sub>N</sub>Ar</b>     | Nucleophilic aromatic substitution   |
| <b>SPhos</b>               | 2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl  |
| <b>sTF<sub>1-219</sub></b> | Soluble tissue factor (residues 1-219)   |
| <b>T</b>                   | Threonine  |
| <b>TAFI</b>                | Thrombin-activatable fibrinolytic inhibitor  |
| <b>TATU</b>                | <i>O</i> -(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium BF <sub>4</sub> <sup>-</sup> |
| <b>TBAF</b>                | Tetrabutylammonium fluoride  |

|  |  |
|--|--|
| <b>TBDMS</b>                           | <i>tert</i> -Butyldimethylsilyl  |
| <b>TBTU</b>                            | <i>O</i> -Benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate         |
| <sup><i>t</i></sup> <b>Bu</b>          | <i>tert</i> -Butyl   |
| <sup><i>t</i></sup> <b>Bu-DavePhos</b> | 2-Di- <i>tert</i> -butylphosphino-2'-( <i>N,N</i> -dimethylamino)biphenyl        |
| <sup><i>t</i></sup> <b>Bu-MePhos</b>   | 2-Di- <i>tert</i> -butylphosphino-2'-methylbiphenyl                              |
| <sup><i>t</i></sup> <b>BuOH</b>        | <i>tert</i> -Butyl alcohol or 2-methyl-2-propanol                                |
| <sup><i>t</i></sup> <b>Bu-XPhos</b>    | 2-Di- <i>tert</i> -butylphosphino-2',4',6'-triisopropylbiphenyl                  |
| <b>TF</b>                              | Tissue factor  |
| <b>Tf</b>                              | Triflate or trifluoromethanesulfonate  |
| <b>TFA</b>                             | Trifluoroacetic acid   |
| <b>Tf<sub>2</sub>O</b>                 | Triflate anhydride   |
| <b>TFMSA</b>                           | Trifluoromethanesulfonic acid  |
| <b>THF</b>                             | Tetrahydrofuran  |
| <b>Thr</b>                             | Threonine  |
| <b>thr</b>                             | Thrombin   |
| <b>TLC</b>                             | Thin layer chromatography  |
| <b>TMS</b>                             | Trimethylsilyl   |
| <b>TMSCI</b>                           | Trimethylsilyl chloride  |
| <b>TMSOTf</b>                          | Trimethylsilyl trifluoromethanesulfonate   |
| <b>TOF</b>                             | Time of flight   |
| <b>Tos</b>                             | Tosyl or ( <i>para</i> -toluenesulfonyl  |
| <b>Trp</b>                             | Tryptophan   |
| <b>trp</b>                             | Trypsin  |
| <b>Trt</b>                             | Trityl or triphenylmethyl  |
| <b>Ts</b>                              | Tosyl or ( <i>para</i> -toluenesulfonyl  |
| <b>TsOH</b>                            | <i>p</i> -Toluenesulfonic acid   |
| <b>TSTU</b>                            | <i>O</i> -( <i>N</i> -Succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate |
| <b>Tween80</b>                         | Polyoxyethylene (20) sorbitan monooleate   |
| <b>Tyr</b>                             | Tyrosine   |
| <b>U</b>                               | Enzyme unit (mass enzyme/ $\mu$ mole substrate converted/minute)                 |
| <b>UC</b>                              | University of California   |
| <b>UCB</b>                             | Union Chimique Belge   |
| <b>UNCA</b>                            | Urethane <i>N</i> -carboxyanhydride  |
| <b>UPLC</b>                            | Ultra Performance Liquid Chromatography  |
| <b>UV</b>                              | Ultraviolet  |
| <b>V</b>                               | Valine   |
| <b>v</b>                               | Enzyme velocity  |
| <b>Val</b>                             | Valine   |
| <b>W</b>                               | Tryptophan   |
| <b>wt.</b>                             | Weight   |
| <b>Xantphos</b>                        | 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene                                  |



**XPhos**  
**Y**

2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl  
Tyrosine



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# Chapter 1

## Design and application of serine protease inhibitors

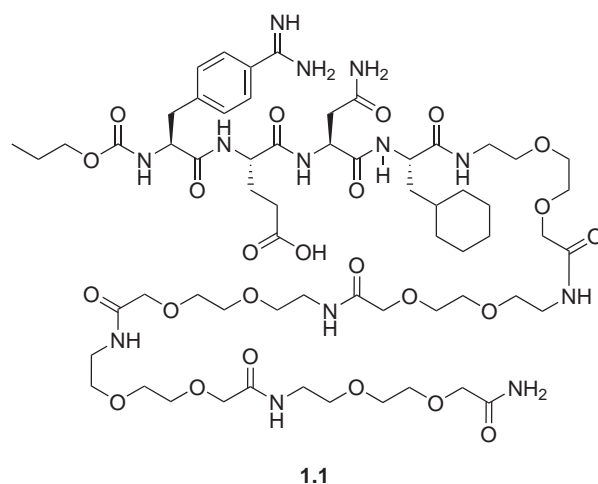
### 1.1 Project introduction

In 1996 Novo Nordisk introduced NovoSeven<sup>®</sup> in Denmark and in 1999 it was launched in the USA. NovoSeven<sup>®</sup> is recombinant activated Factor VII (rFVIIa), a serine protease primarily used for treatment of hemophilia A and B complicated with inhibitors (antibodies).<sup>1,2</sup> Within the last decade, Novo Nordisk has worked within the field of hemostasis and research and development of blood coagulation factors. Currently, several other products are in the R&D pipeline, such as rFVIII, long-acting rFIX and rFXIII for patients undergoing cardiac surgery, a fast-acting rFVIIa and a long-acting rFVIIa.<sup>1</sup>

NovoSeven<sup>®</sup> is administrated intravenously by reconstituting lyophilized rFVIIa in a solvent prior to injection. In 2009 a room temperature stable lyophilized rFVIIa was launched, but an aqueous liquid formulation is still absent primarily due to autoproteolysis of the enzyme.<sup>2</sup> The reconstituted solution is only stable for use for 24 hours at room temperature.<sup>3</sup> FVIIa undergoes degradation by several pathways, including aggregation, oxidation, precipitation and as mentioned also autoproteolysis. Degradation leads to a decreased FVIIa activity thus giving non-homogeneity of dosage, but also a serious risk of increased toxicity and immunogenicity due to formation of protein by-products.<sup>3</sup> Precipitation can lead to thrombosis and clogging of syringes.<sup>3</sup> It would be a great improvement for the patient compliance if a liquid formulation was developed. This would also eliminate reconstitution errors and thereby increase dosage accuracy and simplify the use of the product.<sup>3</sup> A ready-to-use rFVIIa would represent an important improvement of the hemostasis product portfolio of Novo Nordisk.

Protein stability can be affected by many factors, such as ionic strength, pH, temperature and addition of stabilizers. Decrease of activity may be due to chemical factors (pro-

teolysis, deamidation, oxidation, racemization or  $\beta$ -elimination) as well as physical factors (aggregation, precipitation, denaturation or surface adsorption).<sup>4</sup> Novo Nordisk has claimed several peptides<sup>3,5</sup> and small molecules<sup>6-8</sup> in the patent literature as stabilization agents for liquid formulations of rFVIIa. The peptide propyloxycarbonyl-Phe(4-amidino)-Glu-Asn-Cha-(OEG)<sub>5</sub>-NH<sub>2</sub> (**1.1**) was tested in aqueous formulations of 40K-PEGylated rFVIIa (20 mg/mL) at room temperature for 3 months (see Figure 1.1.1).<sup>3</sup> It was found that the best effect of **1.1** was obtained above 1 mM concentrations, e.g. 10 mM of **1.1** retained a rFVIIa activity of 85%. In the absence of the inhibitor, no FVIIa activity was retained at all after 3 months. Furthermore, stabilization might be enhanced by addition of other excipients such as surfactants (e.g. polysorbate or Tween), antioxidants (e.g. ascorbic acid, methionine or cysteine), calcium, magnesium or zinc salts.<sup>9-11</sup> It is also well-known, that proteins can be formulated as liposomes for increased stability.<sup>12-14</sup> However, none of these last-mentioned methods have been reported in attempts to improve the liquid formulations of rFVIIa.



**Figure 1.1.1:** The peptide propyloxycarbonyl-Phe(4-amidino)-Glu-Asn-Cha-(OEG)<sub>5</sub>-NH<sub>2</sub> (**1.1**) was tested as a stabilization agent for rFVIIa formulation. With an inhibitor concentration of 10 mM, a rFVIIa activity of 85% was retained after incubation in 3 months at room temperature.<sup>3</sup>

Addition of an appropriate inhibitor is a useful formulation-aid for stabilization of rFVIIa.<sup>3</sup> The inhibitor should be reversible and sufficient potent to require only minor concentrations present in the final product. However, a highly potent inhibitor is not desirable, because that would completely inhibit rFVIIa and prevent it to initiate blood coagulation clinically. Moreover, the inhibitor should be non-toxic, exhibit favorable solubility in aqueous media and be selective against FVIIa without inhibiting other coagulation factors



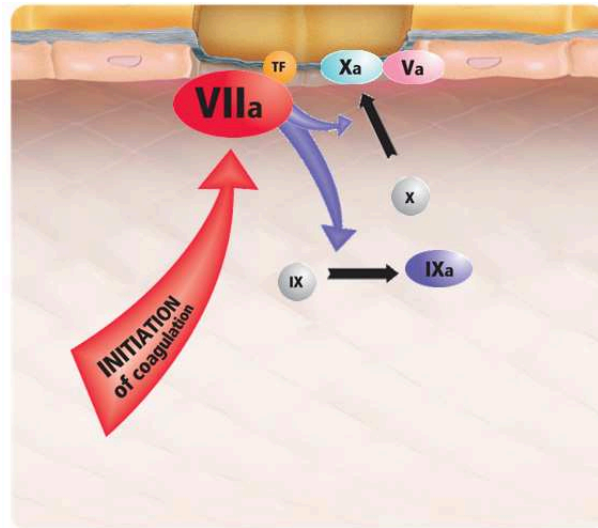
substantially, e.g. thrombin, FXa, FIXa and FXIa.<sup>3</sup> Formulation of rFVIIa containing a stabilizing peptide, should exhibit improved stability and allow prolonged storage, preferably up to 36 months at room temperature.<sup>3</sup>

Concurrently with the development and application of peptide **1.1** toward a liquid formulation of NovoSeven<sup>®</sup>, other peptidyl inhibitors were explored at Novo Nordisk. One of these classes was the peptidyl benzyl ketones which is the topic of this thesis. Benzyl ketones as serine proteases have only been described once in connection with development of thrombin inhibitors, and therefore represent an unexplored class of inhibitors. Selective FVIIa peptidyl inhibitors are in addition rather unknown, as well as probing the S1' binding pocket for achieving potency against FVIIa. Design, synthesis and evaluation of the biological activity of peptidyl benzyl ketones therefore represent an unexplored part of the discovery of potent and selective serine protease inhibitors.

### **1.1.1 Hemostatic control of blood coagulation**

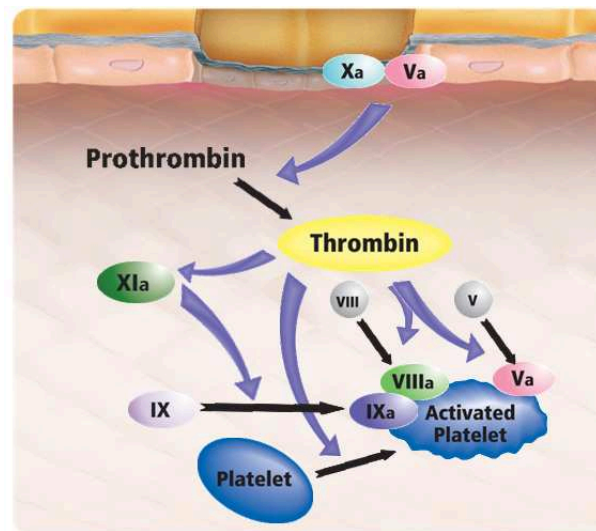
Hemostasis is a highly regulated process that maintains the right viscosity of the blood. It regulates blood coagulation, anticoagulation and fibrinolysis and consists of a combined activity of vascular, platelet and plasma factors.<sup>15–18</sup> Hemostatic abnormalities can lead to uncontrolled bleedings (hemophilia) or abnormal blood clot formation (thrombophilia) in the circulating blood.<sup>19,20</sup> Hemophilia is a group of hereditary genetic disorders, whereas thrombophilia can be a result of an inherited or acquired disease with a higher risk of blood clot formation (thrombosis).<sup>20</sup> Hypercoagulability may also be provoked by drugs to treat hemophilia, surgery, inflammation and in particular atherosclerosis, which is the presence of fatty plaques, such as cholesterol, in the blood. Atherosclerosis is a growing problem due to metabolic syndromes and it is now recognized as a serious public health problem that affects up to 45% of the population (in particular people over 50 years old from USA).<sup>21</sup> Smoking is also known to induce atherosclerosis.<sup>22</sup> The presence of fat in the blood may cause an unpredictable, sudden disruption of fatty plaques which can lead to platelet activation<sup>23</sup> and thrombosis. Abnormal blood coagulation can therefore result in severe intravascular coagulation, venous thrombosis, pulmonary embolism, unstable angina, myocardial infarction or thrombotic stroke. These indications are all being the major causes of morbidity and mortality worldwide.<sup>24</sup>

The blood coagulation is predominantly initiated by a response to trauma, e.g. by injury of a blood vessel.<sup>25</sup> However, the first response, the primary hemostasis, is not the actual blood coagulation, but involves vascular and platelet factors. Local vasoconstriction and compression of injured vessels by extravasation of blood into surrounding tissues are the immediate reactions to injury. Then, vessel walls trigger the attachment and activation of platelets forming aggregates.<sup>25</sup> Simultaneously, the plasma factors are activated mainly



**Figure 1.1.2:** The blood coagulation initiates by exposure of FVIIa from the blood stream to tissue factor (TF) located on cell membranes within and around the blood vessel injury. This leads to activation of FIX and FX.<sup>26–28</sup>

The extrinsic coagulation pathway is initiated by exposure of FVIIa from the circulating blood to tissue factor (TF) located on cell membranes within and around the injury (see Figure 1.1.2).<sup>27,28</sup> The FVIIa-TF complex initiates the secondary hemostasis and a highly regulated cascade of reactions takes place. These events are capable of amplifying a small signal associated with a vessel injury into a major biological event, the formation of a blood clot. FVIIa-TF then activates FIX and FX, and FXa binds to the site of injury with FVa. The amplification phase is then started by conversion of small amounts of prothrombin into thrombin triggered by the FXa-FVa complex.<sup>15-18</sup> Thrombin activates the generation of several activated coagulation factors; FVIIIa, FVa and FXIa and to the further activation of platelets (see Figure 1.1.3).



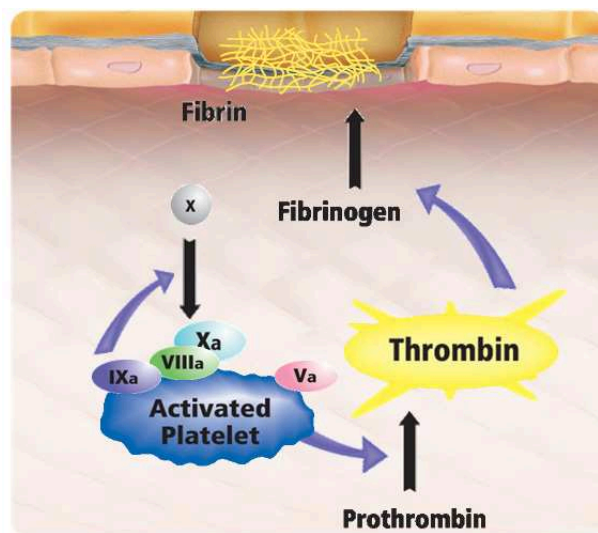
**Figure 1.1.3:** Amplification of the secondary hemostasis starts by formation of small amounts of thrombin, which leads to generation of several activated coagulation factors and activation of platelets.<sup>15–18,26</sup>

The activated platelets then activate FX, and the resulting complex ultimately activates huge amounts of prothrombin generating a thrombin burst - a propagation of the biological event. Thrombin is the last enzyme in the coagulation cascade, and leads to conversion of large amounts of fibrinogen into fibrin. Together with the activated platelets, fibrin forms a hemostatic plug preventing the bleeding to proceed (see Figure 1.1.4).<sup>15–18</sup>

This plug is firm and well-structured and resistant to premature proteolysis and capable of maintaining hemostasis until the wound healing process is established. A full thrombin burst is necessary for the formation of a tight fibrin structure and for the activation of the thrombin-activatable fibrinolytic inhibitor (TAFI), which protects the blood clot against premature proteolysis.<sup>29,30</sup>

These processes are highly regulated by the hemostasis,<sup>25,31</sup> involving several mechanisms and plasma inhibitors, e.g. the heparin-antithrombin (AT) interaction,<sup>32,33</sup> the tissue factor pathway inhibitor (TFPI),<sup>34–36</sup> thrombin-activatable fibrinolytic inhibitor (TAFI),<sup>29</sup> activated protein C,<sup>37</sup> protein S<sup>35,36</sup> and the fibrinolytic systems involving plasmin.<sup>38</sup> Antithrombin (AT) which is a serine protease inhibitor, is the best known due to its important function in the indirect inhibition of thrombin.<sup>33</sup> It degrades thrombin, FIXa, FXa, FXIa and FXIIa and is constantly active. However, its adhesion to these coagulation factors is greatly increased by the presence of heparin, which is a highly sulfated glycosaminoglycan. Heparin is stored within the secretory granules of mast cells and released only into the areas around the tissue injury.  $\text{Ca}^{2+}$  is another important

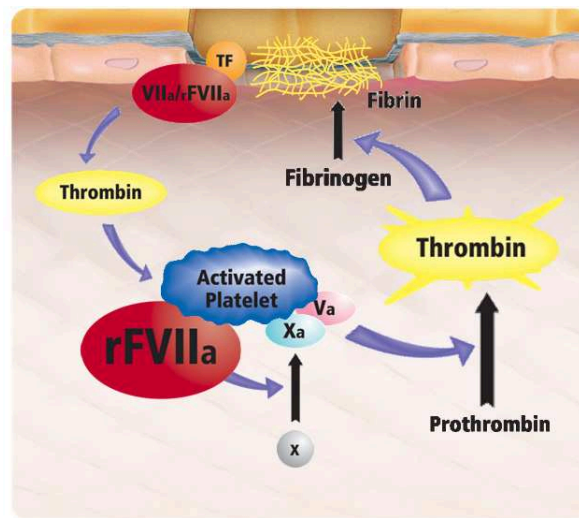
regulator of the secondary hemostasis, because it is involved in the formation of the FVIIa-TF complex and at many other points in the coagulation cascade.<sup>25</sup> The coagulation factors cannot bind to phospholipid surfaces without  $\text{Ca}^{2+}$  being present. Vitamin K is also essential for the hemostasis, because it is responsible for the post-translational  $\gamma$ -carboxylation of glutamic acid residues on thrombin, FVII, FIX, FX as well as on protein C and protein S.<sup>25</sup> Vitamin K-deficiency can therefore lead to acquired hemophilia.



**Figure 1.1.4:** Propagation of the coagulation cascade takes place by forming a complex consisting of activated platelets and several activated factors generating huge amounts of thrombin. This enzyme catalyzes the conversion of fibrinogen to fibrin, which forms the hemostatic plug.<sup>15–18,26</sup>

#### 1.1.1.1 Hemophilia and treatment with NovoSeven<sup>®</sup>

Although thrombophilia is a much more predominant disorder of the hemostasis, hemophilia is still a severe abnormality which is identified as an impair of the ability of the body to control blood coagulation. Around 400,000 males worldwide suffer from hemophilia.<sup>39</sup> Symptoms can be prolonged bleedings, joint bleedings, bleeding in muscles and in subcutaneous tissues and, deep internal bleedings and intracranial bleedings and varing with severity.<sup>39</sup> Hemophilia is a group of recessive sex-linked, X-chromosome disorders, thus only males typically exhibit symptoms. Females have two X-chromosomes and because hemophilia is rare, the chance of a female having two defective copies of the gene is very low. Females are almost exclusively asymptomatic carriers of the disorder.<sup>39</sup>



**Figure 1.1.5:** Exogenous rFVIIa in pharmacological doses binds to the thrombin-activated platelet surface with low affinity, requiring higher concentrations of the blood coagulation factor than those normally found in circulating blood.<sup>26,29</sup>

Approximately 90% of the patients suffering from hemophilia have hemophilia A, which is characterized by a deficiency of FVIII, whereas hemophilia B is a deficiency of FIX.<sup>29,30</sup> Hemophilia causes the formation of loose, fragile fibrin plugs. These blood clots may stop a bleeding initially, but are easily dissolved by proteolytic enzymes, which causes rebleeding repeatedly. Since FVIII or FIX is lacking in the blood circulation, the full thrombin burst does not occur upon FVIIa-TF activation of the coagulation cascade, and the hemostatic plug becomes loose and unstructured. Moreover, TAFI is not fully activated, which leads to premature proteolysis of the blood clot.<sup>29,30</sup>

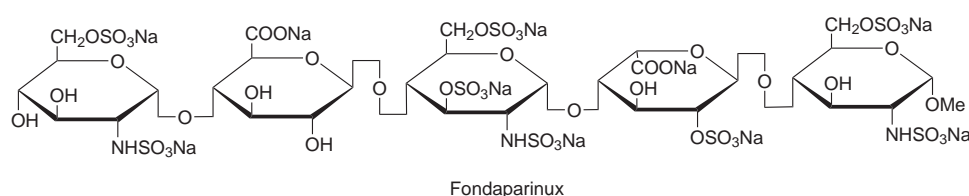
The typical treatment of hemophilia A and B is a replacement therapy, providing the patient with FVIII or FIX, respectively. However, approximately 20% of the patients have developed inhibitors (antibodies) against these unfamiliar coagulation factors.<sup>29</sup> An efficient treatment of those patients was therefore lacking until researchers found, that hemophilia patients infused with FVIIa achieved hemostasis (see Figure 1.1.5).<sup>40</sup> In the late 1980s, Novo Nordisk developed a recombinant FVIIa (rFVIIa) for substitution therapy of hemophilia complicated by inhibitors against FVIII and FIX, with only minimal risk of transmission of infectious agents.<sup>41</sup> The idea was to develop an easily available and convenient treatment of those patients, decreasing the number of bleedings. Moreover, major surgeries on was made possible.<sup>29</sup> Clearance rate and the capacity to generate thrombin on the platelet surface vary widely among individuals. Therefore, the optimal dose is showing great variation in a wider population.<sup>40</sup>

Pharmacological doses of rFVIIa have shown to provide hemostasis in patients with severe hemophilia A and B. This was a breakthrough in the understanding of the importance of FVIIa-TF for hemostasis.<sup>29</sup> rFVIIa binds to the thrombin-activated platelet surface with low affinity, requiring higher concentration of the coagulation factor than those found normally in circulating blood (see Figure 1.1.5).<sup>29</sup> The hemostatic effect of exogenous rFVIIa thus seems to be mediated by an enhanced rate of thrombin generation, resulting in further activation of platelets at the site of injury and fibrin formation.<sup>29</sup>

Treatment with NovoSeven<sup>®</sup> is very safe and no side-effects were observed in healthy volunteers. The incidence of thrombotic events was extremely low, around 1.5% out of 11,000 patients.<sup>29</sup> Almost all of the incidences occurred in non-hemophilia patients with underlying conditions predisposing them to thrombosis. Not all thromboses could be attributed to rFVIIa, and all-cause mortality was 0.3%.<sup>41</sup> No indication of the formation of antibodies against rFVIIa has been observed in patients with hemophilia treated with rFVIIa. However, FVII-deficient patients are at risk for development of antibodies against FVII.<sup>42</sup>

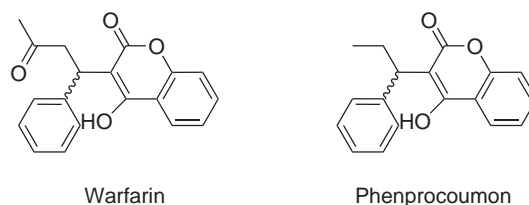
### 1.1.1.2 Regulation of hemostasis indirectly

Many biologically active compounds have been synthesized to regulate the coagulation cascade. The research has predominantly been focusing on inhibitors of thrombin. Thrombin represents an excellent target for antithrombotic therapy, because this enzyme has an important position in the propagation of the coagulation cascade.<sup>32</sup> It is the final enzyme in the cascade and it is the most potent activator of platelets and their aggregation, via the platelet thrombin receptor.<sup>43</sup> The most widely used strategies to prevent thrombosis is by inhibiting generation of thrombin indirectly, either via heparin-like induced activation of AT or by antagonizing the vitamin K-regeneration affecting the post-translational  $\gamma$ -carboxylation of glutamic acid residues on many coagulation factors.<sup>32</sup>



**Figure 1.1.6:** *Fondaparinux, a sulfated glycosaminoglycan, binds to AT with high affinity resulting in an induced activity of AT. This primarily leads to a decreased activation of FX.*<sup>44</sup>

The heparin-like induced activation of AT is typically maintained by low molecular weight heparins (LMWH), e.g. tinzaparin, enoxaparin and fondaparinux which consists of the specific sulfated glycosaminoglycan pentasaccharide known from heparin (see Figure 1.1.6). This sequence binds to AT with high affinity,<sup>45</sup> and increases the inactivation rate of the serine proteases.<sup>46</sup> LMWHs are poorly absorbed when taken orally, thus they have to be administrated parentally. Vitamin K-antagonists are on the other hand orally available, such as warfarin or other coumarin derivatives (see Figure 1.1.7), which are well-known anticoagulants, but they exhibit a slow on-set of action. The risk of prolonged bleedings is the primary risk by using these anticoagulants which is thought to be principally due to their lack of specificity toward numerous serine proteases involved in the blood coagulation.<sup>47</sup>



**Figure 1.1.7:** Warfarin and the coumarin derivative phenprocoumon inhibit the vitamin K-dependent synthesis of the biologically active forms of several coagulation factors and regulatory factor proteins.<sup>48</sup>

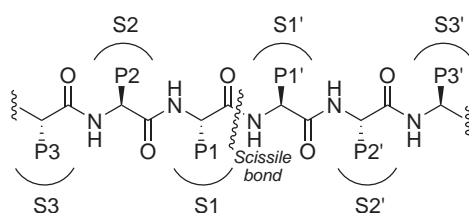
Development of new serine proteases providing better selectivity, potency, pharmacokinetics and oral bioavailability has therefore been a major research topic for several decades.<sup>47</sup> Three-dimensional X-ray crystal structures of the key enzymes in the blood coagulation cascade have aided the design of direct synthetic inhibitors.<sup>49–52</sup> The majority of the inhibitors have been designed for thrombin, whereas the work on the synthesis of potent and selective inhibitors of FVIIa has been limited.<sup>47</sup>

### 1.1.2 Serine proteases and catalytical mechanism

Most of the blood coagulation factors are serine proteases,<sup>18,53</sup> except for a few such as FV and FVIII, which are non-enzymatic glycoproteins. Serine proteases are a group of enzymes that cleave peptide bonds in proteins and they have a characteristic serine in the active site. Closely related enzymes are cysteine and threonine proteases. In mammals, serine proteases evolved by gene duplication to serve functions for example in blood coagulation, digestion, immune system and inflammation. The amide bond cleavage happens indirectly by involvement of a catalytic triad consisting of Ser195, His57 and Asp102 (chymotrypsin numbering).<sup>54,55</sup>



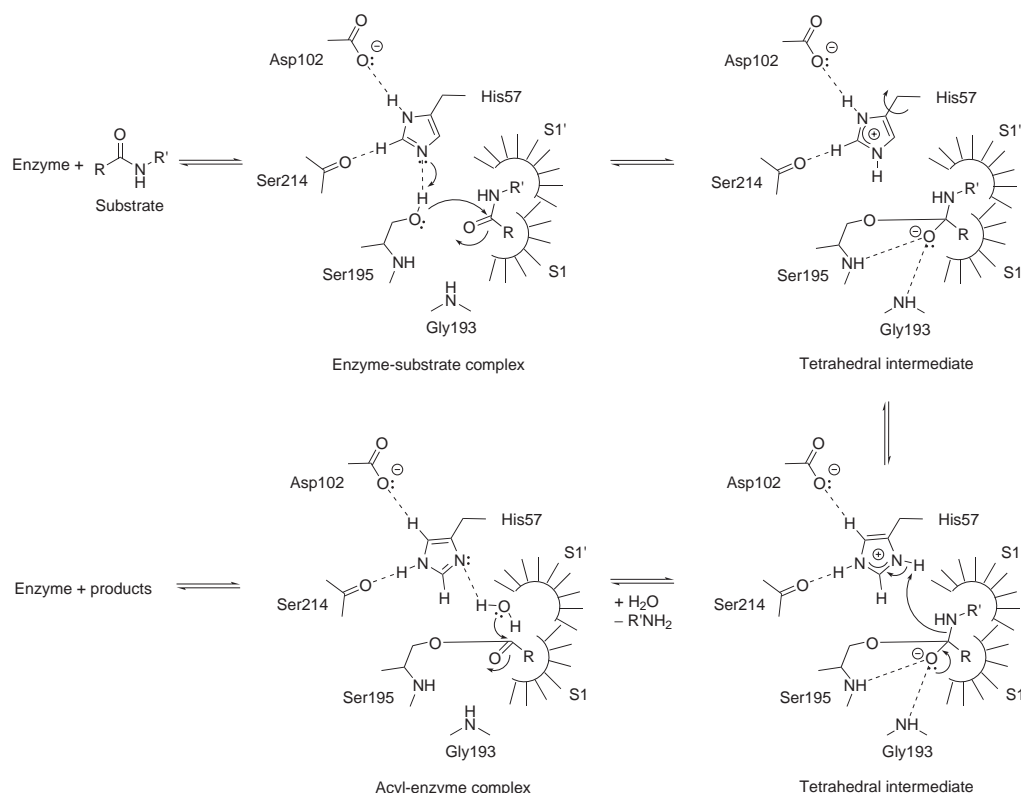
The catalytic mechanism is initiated by a nucleophilic attack from the residue of Ser195, which is facilitated by hydrogen bonding to His57 (see Figure 1.1.9). The resulting tetrahedral intermediate<sup>56</sup> is stabilized by an oxyanion hole formed by the backbone of Ser195 and Gly193.<sup>54,55</sup> Elimination of the amine product occurs by flip of His57,<sup>57</sup> proton donation and formation of an acyl-enzyme complex.<sup>58</sup> Water occupies the vacant S1' binding pocket, adds to the acyl enzyme complex and generates a carboxylic acid by elimination through another tetrahedral intermediate<sup>56</sup> and His57 ring flip<sup>57</sup> (not shown on Figure 1.1.9).



**Figure 1.1.8:** Nomenclature of the active site of a serine protease.<sup>59</sup>  $P_n$  designate amino acid residues of the peptide substrate, whereas  $S_n$  designate the corresponding binding sites. The scissile bond is  $P1-P1'$ .

The specificity of serine proteases arises from the binding pockets (sites) ( $S_n$ ) positioned on both sides of the scissile bond. Each of these substrate binding pockets are able to accommodate complementary specific amino acid residues ( $P_n$ ) (see Figure 1.1.8).<sup>59</sup>



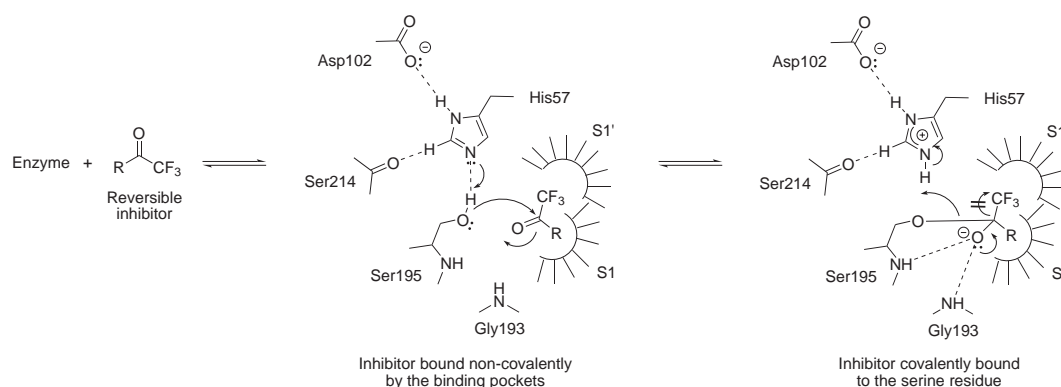


**Figure 1.1.9:** The catalytic mechanism of a serine protease initiates by formation of an enzyme-substrate complex. Through a tetrahedral intermediate the amine product is eliminated first, then, after addition of water to an acyl-enzyme complex, the carboxylic acid product is also eliminated.<sup>54,55</sup>

### 1.1.3 Inhibitors of serine proteases

Enzyme inhibitors can be classified as competitive or non-competitive as well as reversible or irreversible.<sup>60</sup> Competitive inhibitors bind to the active site of the enzyme, whereas non-competitive inhibitors bind to other sites thus resulting in an allosteric regulation of the enzyme leading to conformational changes.<sup>60</sup>

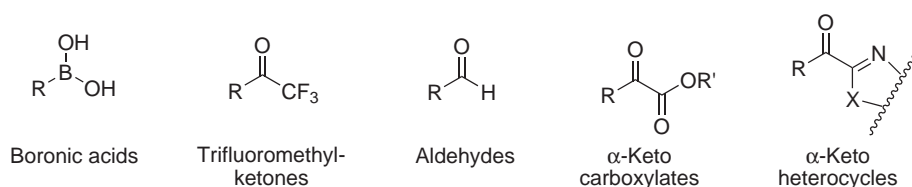
Competitive inhibitors of serine proteases strongly resemble the natural substrate and therefore the compounds compete with each other for binding to the active site. Reversible inhibitors can bind to the active site covalently or non-covalently, however irreversible inhibitors of serine proteases always bind covalently. Reversible, covalently bound inhibitors react with Ser195 in the active site, thus forming a covalent bond.<sup>54</sup> Irreversible inhibitors also alkylate His57 by elimination of a good leaving group from



**Figure 1.1.10:** Binding of a competitive reversible inhibitor to a serine protease. The R-group is the rest of the inhibitor molecule which is embedded non-covalently to the binding pockets of the enzyme.<sup>54</sup> The trifluoro ketone inhibitor illustrated here is a reversible inhibitor because -CF<sub>3</sub> and -F are poor leaving groups.

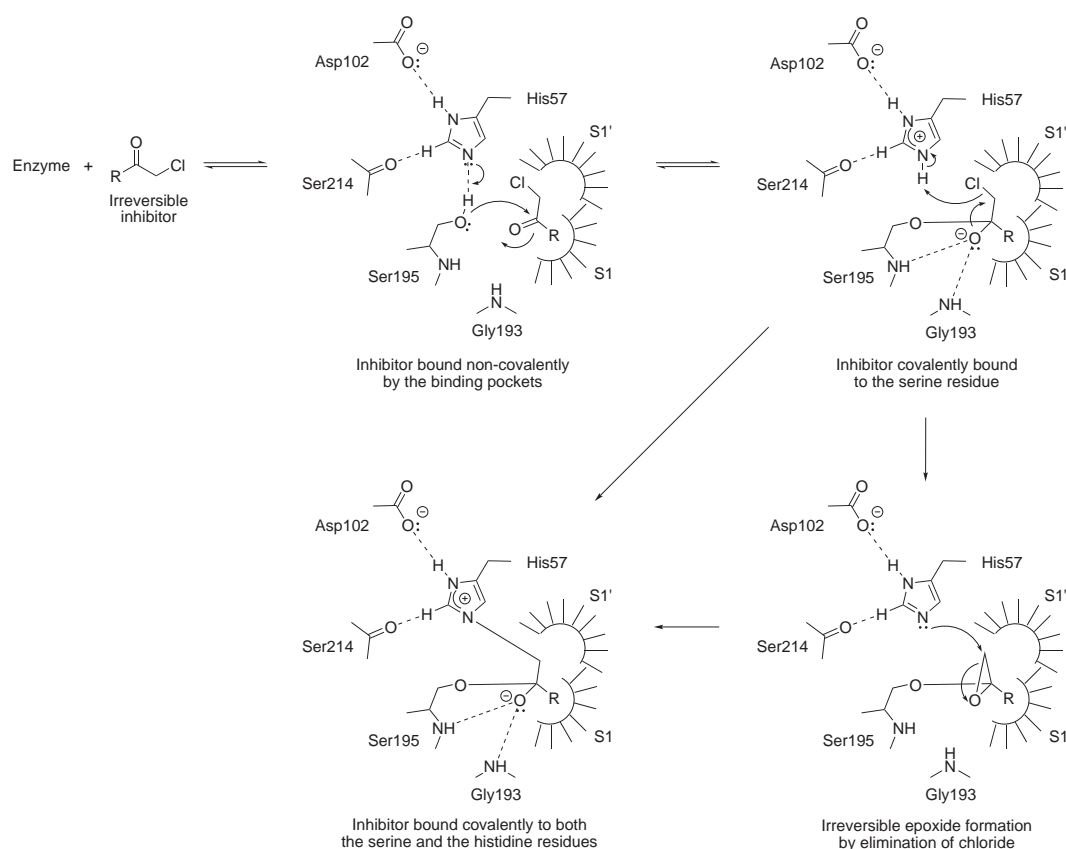
the inhibitor molecule. In the absence of such a leaving group, the inhibitor becomes reversible in nature (see Figure 1.1.10).

In general, molecules containing a neutral electrophilic group (serine trap) in the P1–P1' position such as a boronic acid, a trifluoromethyl ketone, an aldehyde, an  $\alpha$ -keto carboxylate or an  $\alpha$ -keto heterocycle, are potential inhibitors of serine proteases (see Figure 1.1.11). Several reviews on synthetic and natural inhibitors containing one of these functionalities exist in the literature.<sup>61,62</sup>



**Figure 1.1.11:** Molecules containing an electrophilic functionality (serine trap) in the P1–P1' position such as boronic acids, trifluoromethyl ketones, activated ketones and aldehydes are potential competitive, reversible inhibitors of serine proteases.<sup>54</sup>

As shown in Figure 1.1.10, Ser195 forms a covalent bond with the inhibitor generating a hemiacetal. The resulting negative charge is stabilized by the oxyanion hole, whereas the positive charge on His57 is stabilized by Asp102 making the process favorable.<sup>54</sup>



**Figure 1.1.12:** Chloromethyl ketones (cmk) are well-known irreversible inhibitors of serine proteases. After formation of a hemiacetal with Ser195, His57 is forming a covalent bond with the inhibitor molecule, either directly<sup>63</sup> or through formation of an epoxide.<sup>64</sup> The alkylation of His57 is an irreversible reaction.

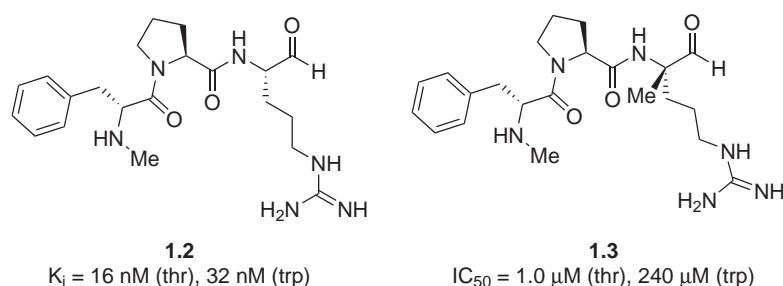
The inhibitory process is quite similar for the irreversible protease inhibitors (see Figure 1.1.12). It is the nucleophilic and the basic residues of the catalytic triad which are targeted by these compounds.<sup>54,65</sup> One of the most classical active site-directed irreversible inhibitors of serine proteases is the peptidyl chloromethyl ketones (cmk).<sup>65–67</sup> These compounds alkylate His57 in the active site thus providing the irreversible nature. Due to the inherent unreactivity of carbon-fluorine bonds, peptide fluoromethyl ketones are reversible inhibitors of serine proteases (c.f. Figure 1.1.10).<sup>65</sup> Chloromethyl ketones are believed to inhibit the serine proteases by first forming a hemiacetal with Ser195,

and then either a direct substitution takes place forming a covalent bond with His57<sup>63</sup> or an indirect substitution through the formation of an epoxide.<sup>64</sup>

Chloromethyl ketones (cmk) are potent alkylating agents and absolute specificity with a particular protease would be difficult to achieve.<sup>65</sup> Because of their potential toxicity that results from non-selective alkylation of cellular nucleophiles, peptidyl chloromethyl ketones have very little biological utility. However, they have been used as tools *in vivo* to identify whether a particular serine protease inhibitor might have a therapeutic effect on a disease state or in animal models.<sup>65</sup> Other potential irreversible inhibitors of serine proteases are compounds containing e.g. carbamate esters,<sup>68–72</sup> *O*-acylhydroxylamines,<sup>73–79</sup>  $\beta$ -lactams,<sup>80</sup> isocoumarins,<sup>81–83</sup> benzoxazin-4-ones,<sup>84,85</sup> saccharins,<sup>86,87</sup> sulfonyl fluorides<sup>88</sup> and many other functionalities that are more specific toward cysteine proteases.<sup>65</sup>

### 1.1.3.1 Thrombin inhibitors

Because of the important role in the blood coagulation cascade, many inhibitors of thrombin have been developed. In general, these inhibitors have been designed as structural variations of the P4–P1' positions as peptides or peptidomimetics. Using peptidomimetics has the goal to diminish as much as possible of their peptidic character in order to avoid *in vivo* hydrolysis and improve bioavailability, e.g. by substituting the basic arginine with a less basic surrogate,<sup>55</sup> or by converting the amidine group into a prodrug, e.g. an alkoxy-amidine, a carbamate<sup>89,90</sup> or a *N,N'*-dihydroxyamidine,<sup>91</sup> which will reduce basicity and polarity and hence improve the pharmacokinetics.

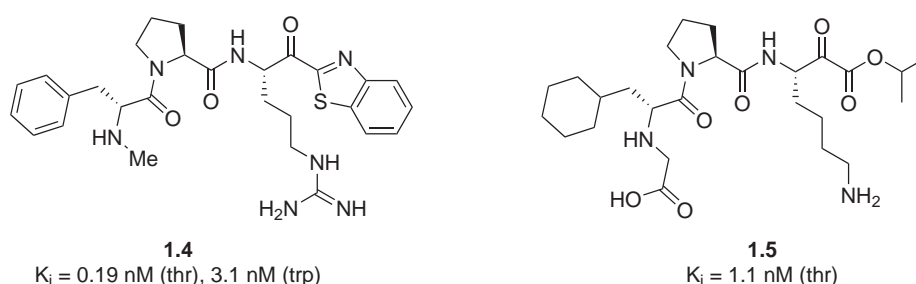


**Figure 1.1.13:** Tripeptide aldehydes, such as **1.2**<sup>92</sup> and **1.3**<sup>93</sup> with the general *D*-Phe-Pro-Arg structure were among the first synthetic reversible thrombin inhibitors.

Decades ago it was discovered that leupeptins are reversible inhibitors of serine proteases. Leupeptins are C-terminal aldehydes with an acetylated or propionylated *N*-terminal, e.g.

Ac-Leu-Leu-DL-Arg-H and analogs in which Leu is replaced with Val or Ile.<sup>94</sup> These compounds are produced by various species of actinocyete bacteria.<sup>95</sup> Later on, it was found that H-D-Phe-Pro-Arg-cmk was a selective affinity label for thrombin<sup>96</sup> and inspired by these results, Bajusz *et al.* reported that *N*-Me-D-Phe-Pro-Arg-H (**1.2**) is a highly active, reversible inhibitor of thrombin,<sup>97</sup> however not selective against trypsin (trp)<sup>92</sup> (see Figure 1.1.13). Introducing an  $\alpha$ -methyl group at the P1 position resulted in **1.3**, which showed a decreased potency against thrombin, but on the other hand exhibited an improved selectivity against trypsin and other related serine proteases such as plasmin and FXa.<sup>93</sup> This clearly illustrates the challenge in designing protease inhibitors; potency and selectivity are indeed not always optimized at the same time.

The potency of the peptidyl sequence in **1.2** was improved by substituting the aldehyde C-terminal functionality with a trifluoromethyl group, which gave  $K_i < 1$  nM against thrombin.<sup>98</sup> Apparently, the aldehyde carbonyl is less electrophilic than the trifluoromethyl ketone carbonyl. One of the most potent synthetic peptidyl thrombin inhibitor is the H-D-Phe-Pro-boroArg-OH with  $K_i = 41$  pM.<sup>99</sup> Specificity for the S1 binding pocket was examined with different P1 residues, such as ornithine, lysine and homolysine but none of them were superior to the arginine derivative.<sup>100</sup> However, potencies in the nanomolar range were still observed.



**Figure 1.1.14:** Examples of other reversible thrombin inhibitors; **1.4**<sup>101</sup> which is a 2-benzothiazole and **1.5**<sup>102</sup> which is an  $\alpha$ -keto isopropyl carboxylate derivative both demonstrating high potencies.

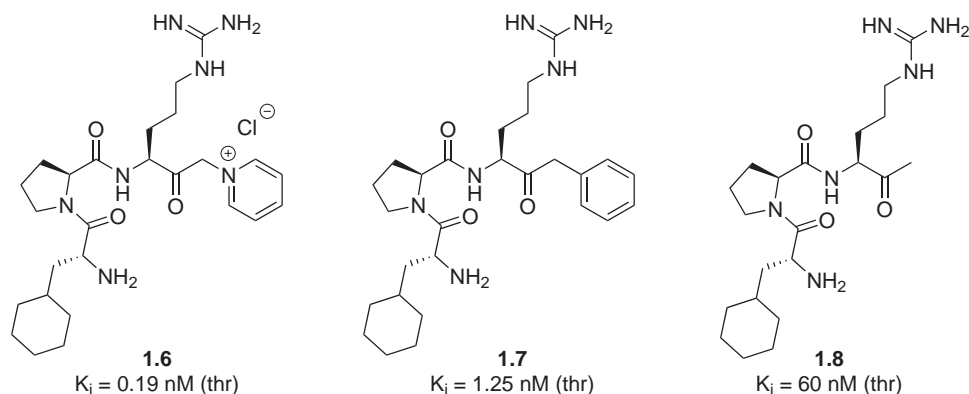
Synthesis of peptidyl  $\alpha$ -ketobenzoxazoles by Strimpler and co-workers in 1992 was the first example of a carbonyl activated by a heterocycle; Ac-Val-Pro-Val-(2-benzoxazole) as an inhibitor of elastase. Furthermore, it was the first example of an inhibitor designed to interact with both Ser195 and non-covalently with His57 in the active site.<sup>103</sup> From the X-ray structure it was found that nitrogen from the benzoxazole group interacted with the protonated His57. These prior results were used for development of potent thrombin inhibitors proping the binding pocket near S1' as a novel interaction site with thrombin.<sup>97</sup> The best inhibitor was found to be *N*-Me-D-Phe-Pro-Arg-(2-benzothiazole)

(**1.4**) with  $K_i = 0.19$  nM (see Figure 1.1.14).<sup>101</sup>

A variety of different heterocycles were tested, among others 2-thiazole, 2-benzoxazole, 2-pyridine and *N*-Me-2-imidazole, but none of them were as potent as **1.4**. Once again, selectivity for trypsin was poor. It seems to be a general property of inhibitors with the D-Phe-Pro-Arg motif.<sup>101</sup> However, the heterocycle was shown to exhibit key interactions near and in the S1' region of the active site of thrombin. The benzothiazole ring forms a hydrogen bond with His57 and an aromatic stacking interaction with a tryptophan residue originating from the unique insertion loop of thrombin.<sup>101</sup> An in-depth study of this class of thrombin inhibitors utilizing the S1' binding pocket has been reported.<sup>104</sup> The study revealed that the preferred  $\alpha$ -ketoheterocycle was a  $\pi$ -rich 2-substituted azole with at least two heteroatoms proximal to the carbon bearing the keto group. The 2-benzothiazole was unfortunately found to cause hypertension and electrocardiogram abnormalities in animals.<sup>104</sup> Therefore, a 2-benzothiazole-6-carboxylic acid derivative of **1.4** was developed showing a much better therapeutic profile. The carboxylic acid was found to form a salt bridge with Lys192.<sup>104</sup>

In the search for orally available thrombin inhibitors, it was found that the  $\alpha$ -keto acid H-D-Phe-Pro-Lys-CO<sub>2</sub>OH was potent with  $K_i = 1.4$  nM.<sup>102</sup> Through lead optimization Adang *et al.* discovered **1.5** (see Figure 1.1.14) as another potent inhibitor of thrombin ( $K_i = 1.1$  nM) with improved pharmacokinetics, such as increased half-life and bioavailability.<sup>102</sup>

To elucidate whether a high potency is a result of electron withdrawing effects giving a electrophilic carbonyl or enhanced hydrophobic interactions in the S1' region with the aromatic moiety, Steinmetzer and Konishi performed a small comparative study.<sup>105</sup> Three different thrombin inhibitors with the general structure H-D-Cha-Pro-Arg were tested; a peptidyl pyridinium methyl ketone **1.6** containing a strongly electron deficient carbonyl and a hydrophobic moiety in the P1' region, a peptidyl benzyl ketone **1.7** containing only the hydrophobic moiety and a peptidyl methyl ketone **1.8** containing neither of the properties (see Figure 1.1.15).



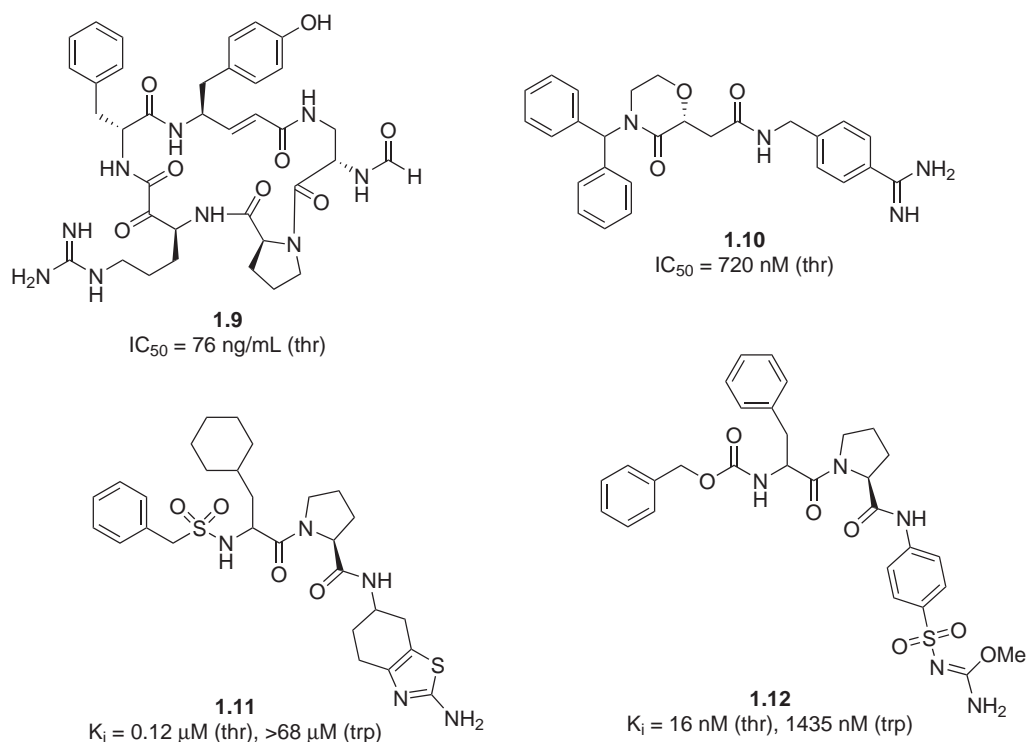
**Figure 1.1.15:** Comparative study of *H-D-Cha-Pro-Arg* thrombin inhibitors revealed that both electronic withdrawing effects and hydrophobicity was important for high potency against thrombin.<sup>105</sup>

The results clearly indicated that the high potency is based on both electron withdrawing and hydrophobic effects.<sup>105</sup> Compared to the peptidyl pyridinium methyl ketone **1.6**, the benzyl ketone inhibitor **1.7** lacking the permanent positive charge, had a ten-fold reduced inhibitory activity. However, **1.7** is still a potent thrombin inhibitor. A much more pronounced decrease in activity was observed with the peptidyl methyl ketone **1.8**, which had a  $K_i = 60 \text{ nM}$ .<sup>105</sup> It seems that hydrophobic interactions with the P1' moiety are of greater importance than the electrophilicity of the carbonyl group.<sup>105</sup>

The tripeptide scaffold has accounted for many other thrombin inhibitors, containing electrophilic serine traps such as  $\alpha$ -hydroxy and  $\alpha$ -ketoesters,<sup>106</sup> amide and  $\alpha$ -keto carbonyls,<sup>107</sup> phosphonic acid esters<sup>108</sup> and  $\beta$ -lactams.<sup>109,110</sup> Moreover, ketomethylene pseudopeptides<sup>111</sup> and macrocyclic peptides, e.g. cyclotheonamide A (**1.9**)<sup>112,113</sup> (see Figure 1.1.16), have been found to be potent inhibitors of thrombin.

To improve the selectivity and other pharmacokinetic properties, many small molecules have been synthesized lacking the electrophilic serine trap.<sup>55</sup> These molecules inhibit thrombin in a non-covalent manner thus decreasing in activity. With careful structure-activity relationship studies and lead optimization potent inhibitors have been designed, e.g. the morpholinone-based benzamidine analog **1.10**,<sup>114</sup> the heterocyclic arginine mimetic **1.11**,<sup>115,116</sup> the Cbz-protected non-basic sulfonyl-*O*-methylisourea **1.12**<sup>117</sup> (see Figure 1.1.16) and many others.<sup>53,55,118–120</sup>

Despite the immense numbers of thrombin inhibitors reported in the literature, the development of new compounds is still ongoing today.<sup>121,122</sup> Unfortunately, very few of the inhibitors possess all of the required properties regarding potency, selectivity and

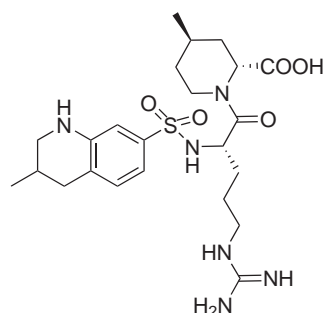


**Figure 1.1.16:** A variety of different thrombin inhibitors; Cyclotheonamide A (**1.9**)<sup>112,113</sup> and three non-covalent binding peptidomimetics **1.10–1.12**.<sup>114–117</sup> The latter shows enhanced bioavailability due to the less basic arginine-mimetic functionality.

pharmacokinetic properties to be used as an antithrombotic agent.

As of 2009, several direct inhibitors of thrombin (thr) and FXa are in clinical trials,<sup>123</sup> including compounds from pharmaceutical companies such as AstraZeneca, Lilly, Bristol-Myers Squibb and Sanofi-Aventis. Only a few serine protease inhibitors have entered the market, one of them is argatroban (see Figure 1.1.17), which is an inhibitor of thrombin,<sup>124,125</sup> administered parenterally and used as an anticoagulant.<sup>120</sup>





Argatroban  
 $K_i = 39 \text{ nM (thr)}$

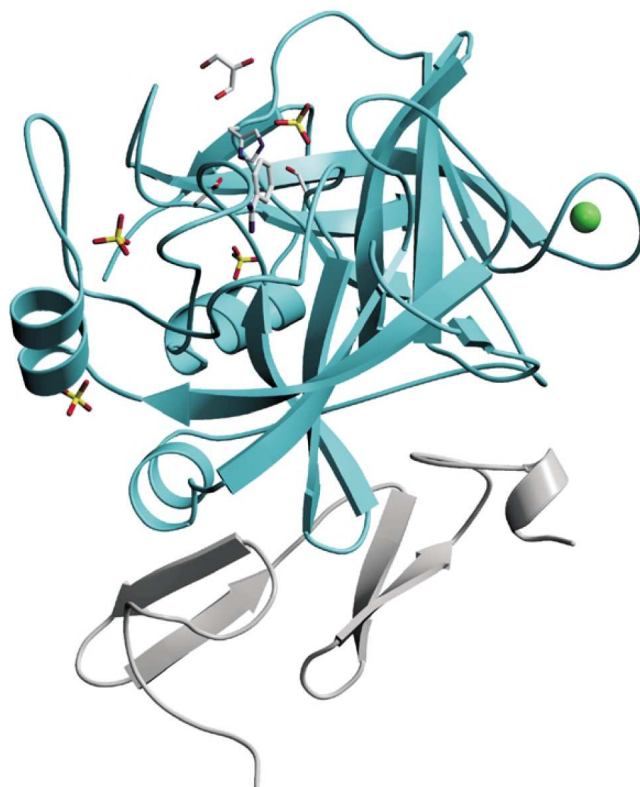
**Figure 1.1.17:** Argatroban is one of the few serine protease inhibitors on the market.<sup>120</sup> It is a potent reversible thrombin inhibitor with  $K_i = 39 \text{ nM}$ ,<sup>124,125</sup> but with low bioavailability and thus has to be administrated parenterally.

### 1.1.3.2 Structural distinctiveness of FVIIa

In the circulating blood FVII exists as a zymogen consisting of an amino-terminal  $\gamma$ -carboxy glutamic acid-rich (Gla) domain, followed by two epidermal growth factor (EGF)-like domains, a shorter linker peptide and a carboxy-terminal serine protease domain.<sup>51</sup> The active enzyme, FVIIa, is generated by specific cleavage of the peptide bond between Arg15 and Ile16 at the end of the linker peptide, giving a *N*-terminal light chain of 152 residues and a heavy chain of 254 residues linked by a disulphide bridge. FVIIa does only reach its full enzymatic potential when complexed to TF in the presence of  $\text{Ca}^{2+}$  ions.<sup>51</sup> The catalytic domain and the EGF2-domain are shown in Figure 1.1.18.

Although the serine proteases have a highly homologous three-dimensional structure<sup>127</sup> and the catalytic domain of FVIIa has a core structure common within the thrombin/trypsin family,<sup>51</sup> they display significant differences in specificity and catalytic activity. A number of surface loops defines the nature and extent of the hydrophobic substrate/inhibitor binding pockets because of the varying lengths and compositions of the loops.<sup>127</sup> These differences result primarily in different S3 and S2 binding pockets. Contrarily, the S1 pocket is very similar among the serine proteases such as FVIIa, thrombin and FXa. However Ser190 positioned in S1 is unique for FVIIa.<sup>128</sup> In addition, FVIIa contains a unique primary structure; the 170-loop which has five extra amino acid residues compared to related proteases. This loop is close to the TF-contacting surfaces and is important for the FVIIa interaction with TF.<sup>127</sup>

Comparing the S3 and S2 binding pockets of FVIIa with thrombin and FXa reveals several differences. First of all, FVIIa has a relatively large, hydrophilic and negatively



**Figure 1.1.18:** The catalytic domain (blue) and the EGF2-domain (grey) of FVIIa complexed with benzamidine. Sulphate groups and glycerol moieties are also shown (ball and sticks) as well as  $\text{Ca}^{2+}$  ions (green).<sup>126</sup>

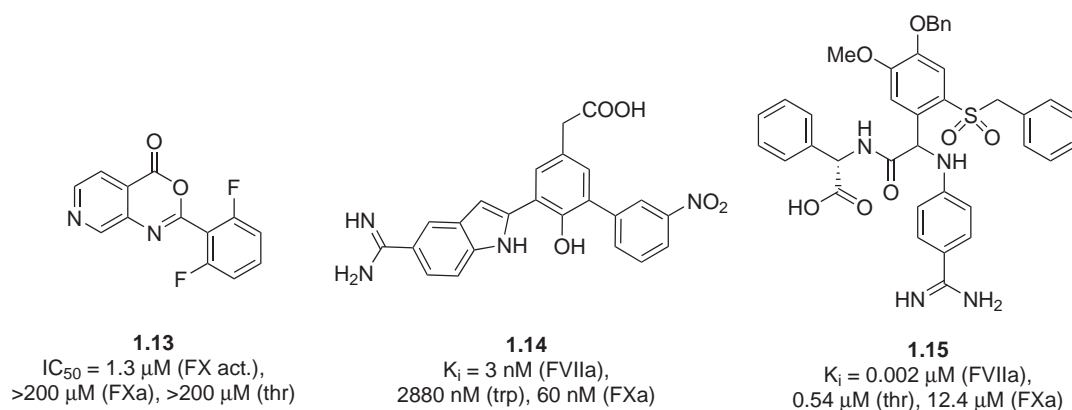
charged S2 pocket due to the unique presence of Asp60.<sup>129</sup> Secondly, the S3 pocket is small consisting of several amino acids including Gln217<sup>130</sup> and thirdly, the unique 170-loop is just long enough to interfere with substrate binding in S3.<sup>51</sup> Contrarily, thrombin has a hydrophobic S2 with bulky substituents,<sup>131–133</sup> and a large and hydrophobic S3 pocket.<sup>132</sup> Utilizing the unique structural features of FVIIa would be advantageous in gaining selectivity against other serine proteases.<sup>130</sup> Selectivity could be introduced by inhibitors interacting with Asp60, Ser190, Lys192, Gln217, the 170-loop,<sup>130</sup> but also by varying the sizes of P3 and P2 residues, e.g. introducing a small group at the P3 position.

Structure-based inhibitor design to achieve potent and selective inhibitors of FVIIa-TF was initiated by X-ray crystal structures of FVIIa-TF complexed with different inhibitors. Structure determination of FVIIa-TF was pioneered by Banner *et al.* in 1996 reporting the X-ray structure of FVIIa-TF in complex with H-D-Phe-Phe-Arg-cmk.<sup>51</sup> Besides of structure-based design, new FVIIa inhibitors have been designed from high throughput

screening (HTS) results,<sup>134</sup> or as analogs from thrombin/FXa inhibitor libraries.<sup>135,136</sup>

### 1.1.3.3 FVIIa inhibitors

Development of specific FVIIa inhibitors became an interesting field of research, when problems with inhibition of the late-stage coagulation enzymes were discovered, e.g. inhibition of thrombin or FXa. One of the major problems with direct thrombin inhibitors as anticoagulants is prolonged bleedings. Inhibition of the coagulation cascade at its initial stage by inhibition of FVIIa-TF was found to be an alternative and promising approach for the development of new anticoagulants. This observation was confirmed by several animal models.<sup>137–141</sup> However, inhibiting FVIIa to gain anticoagulation is on the other hand challenging, because only minor amounts of this enzyme is required for initiation of the blood coagulation cascade.

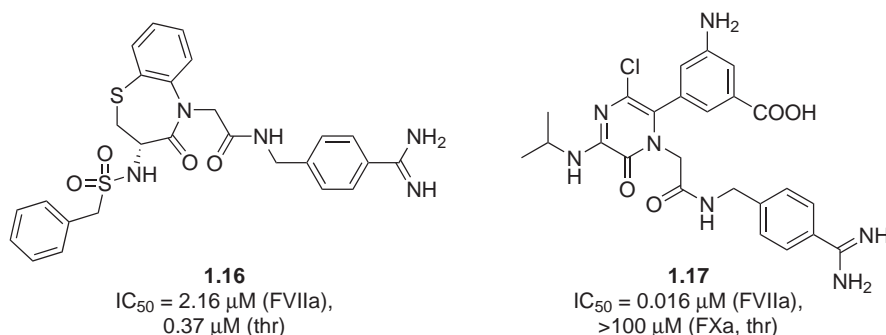


**Figure 1.1.19:** A variety of small molecule reversible FVIIa inhibitors; **1.13** is a pyridine analog of a 2-aryl substituted hetero annulated 1,3-oxazin-4-one,<sup>135</sup> **1.14** is a 2-substituted 5-amidinobenzoindole<sup>142</sup> and **1.15** is a phenylglycine analog.<sup>143,144</sup> FX act.: Inhibitory effect against FVIIa measured indirectly as degree of FX activation.

The number of reported FVIIa-TF inhibitors is rather limited, compared to the number of thrombin inhibitors. The vast majority of the reported inhibitors are small molecules that do not covalently bind to the active site. These compounds show superior pharmacokinetics, such as improved half-life and bioavailability. Some of the first FVIIa inhibitors were reported 10 years ago by Novo Nordisk<sup>135,145</sup> (e.g. **1.13**, see Figure 1.1.19), Novartis Pharmaceuticals<sup>134</sup> and Ono Pharmaceuticals.<sup>146</sup>

Among the FVIIa small molecule inhibitors, compound classes such as amidinonaphthols,<sup>134</sup> 2-aryl substituted hetero annulated 1,3-oxazin-4-ones (e.g. the pyridine analog **1.13**),<sup>135</sup> indolizidinones,<sup>147</sup> dysinosin A (aeruginosins),<sup>148,149</sup> pyrazinones,<sup>150–153</sup> amidophenylureas,<sup>154</sup> 2-pyridones,<sup>155,156</sup> 2-substituted 5-amidinobenzoindoles (e.g. **1.14**),<sup>142,157</sup> fluorobenzenes,<sup>158</sup> benzoquinones,<sup>158</sup> 2-substituted-4-amidinophenylpyrivic acids,<sup>136</sup> fluoropyridines,<sup>159</sup> phenylglycines (e.g. **1.15**),<sup>143,144</sup> non-amidine leucine amides,<sup>160</sup> 5-aminopyrrolo[3,2-b]pyridines,<sup>161</sup> 1,5-benzothiazepine-4-ones,<sup>162</sup> benzoimidazole succinic acids<sup>163</sup> and biphenyls<sup>129,157</sup> have been reported. Genereally, FVIIa inhibitors incorporate a basic P1 guanidino, amidino or amino group which interacts with Asp189 in the S1 binding pocket.

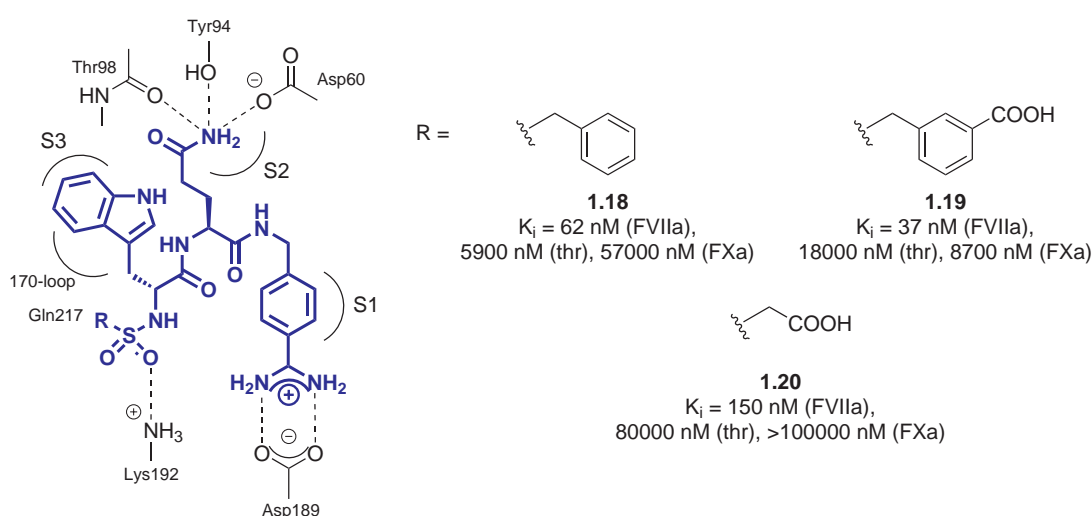
Recently, Ayral *et al.*<sup>162</sup> reported the design and synthesis of 1,5-benzothiazepine-4-ones with a benzyl sulfonyl P3 residue as FVIIa-TF inhibitors. They found that **1.16** was the best inhibitor with a  $IC_{50} = 2.16 \mu M$  against FVIIa-TF, but with poor selectivity against thrombin (see Figure 1.1.20). Four different P3 residues were explored; succinyl ( $81.0 \mu M$ ), acetyl ( $90.0 \mu M$ ),  $HOOC-CH_2-$  ( $15.9 \mu M$ ) and  $Ph_2CHCO-$  ( $33.0 \mu M$ ), but all of them gave lower potencies ( $IC_{50}$ -values stated in brackets).<sup>162</sup> X-ray crystallography revealed that the benzylsulfonyl group in **1.16** pointed outside S3 and was close to S4, making a hydrophobic interaction with the side-chain of Gln217.<sup>162</sup>



**Figure 1.1.20:** The 1,5-benzothiazepine-4-one **1.16** was found to be the best inhibitor in the group of 1,5-benzothiazepine-4-ones designed and synthesized by Ayral *et al.*,<sup>162</sup> whereas Parlow and co-workers found that the *N*-isopropyl derivative **1.17** was the best inhibitor among a variety of pyrazinones.<sup>151</sup>

The Parlow group has also explored alternative *N*-terminal-like modifications. For the synthesis of pyrazinones as FVIIa inhibitors,<sup>151</sup> it was found that the *N*-isopropyl derivative **1.17** was the best, whereas  $PhCH_2CH_2-$  ( $0.62 \mu M$ ), which resembles the benzylsulfonyl hydrophobic part, had a decreased potency against FVIIa.<sup>151</sup>

Shiraishi *et al.*<sup>130</sup> reported in 2009 a range of peptidomimetics with a glutamine moiety at P2, a D-tryptophan moiety at P3 and a *N*-terminal sulfonyl functionality at P4. This resulted in a series of potent FVIIa inhibitors **1.18–1.20** (see Figure 1.1.21). Three different P4 substituents were tested; the benzyl sulfonyl **1.18**, the *meta*-carboxyl benzyl sulfonyl **1.19** and the carboxyl methyl sulfonyl **1.20**, and all of them were potent FVIIa inhibitors with **1.19** being the best. Compared to the benzyl sulfonyl **1.18**, **1.19** showed an increased selectivity against thrombin, but decreased selectivity against FXa. The most selective inhibitor was the carboxyl methyl sulfonyl **1.20**, which on the other hand showed less potency against FVIIa.<sup>130</sup>



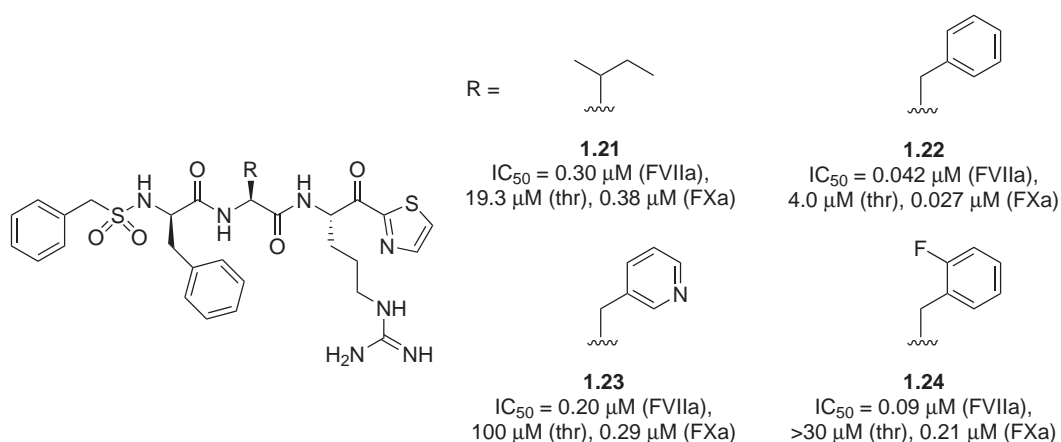
**Figure 1.1.21:** Potent peptidomimetic inhibitors of FVIIa exploring different *N*-sulfonylation terminals and the binding mode of the best inhibitor.<sup>130</sup>

A crystal structure of FVIIa-TF bound to **1.20** shows the typical binding modes in S1 forming a salt bridge with Asp189 (see Figure 1.1.21). Moreover, the P2 glutamine probed the S2 binding pocket with unique hydrogen bonds to Thr98, Tyr94 and Asp60. P3 D-tryptophan occupies the S3 site and has large contact areas with Gln217 and the unique 170-loop residues. The sulfonyl group forms a long-distance (4.2 Å) ionic interaction with Lys192 in the sub site of the S1 binding pocket. Lys192 is not conserved among the proteases, and only FVIIa and FXIa have Lys192 at this position.<sup>130</sup> The carboxyl methyl substituent in **1.20** extends to the direction of the S4 site, but it does not reach it (not shown in Figure 1.1.21). In the case of the presence of a benzyl sulfonyl group, van der Waals contracts with a number of amino acids in the subsite of the S1 binding pocket exists, and by introduction of the *meta*-carboxylic acid ionic interactions were observed with Lys192.<sup>164</sup> Lys192 has also been reported making favorable interactions in the S1' pocket with a 2-hydroxyphenyl P1' substituent.<sup>157</sup> The sudden occupancy of

Lys192 near the oxyanion hole relays on a 180°-rotation, which is unique for FVIIa and is due to hydrogen bonding to Gln143.<sup>157</sup> Probing S1' is thus another way to develop selective and potent inhibitors.

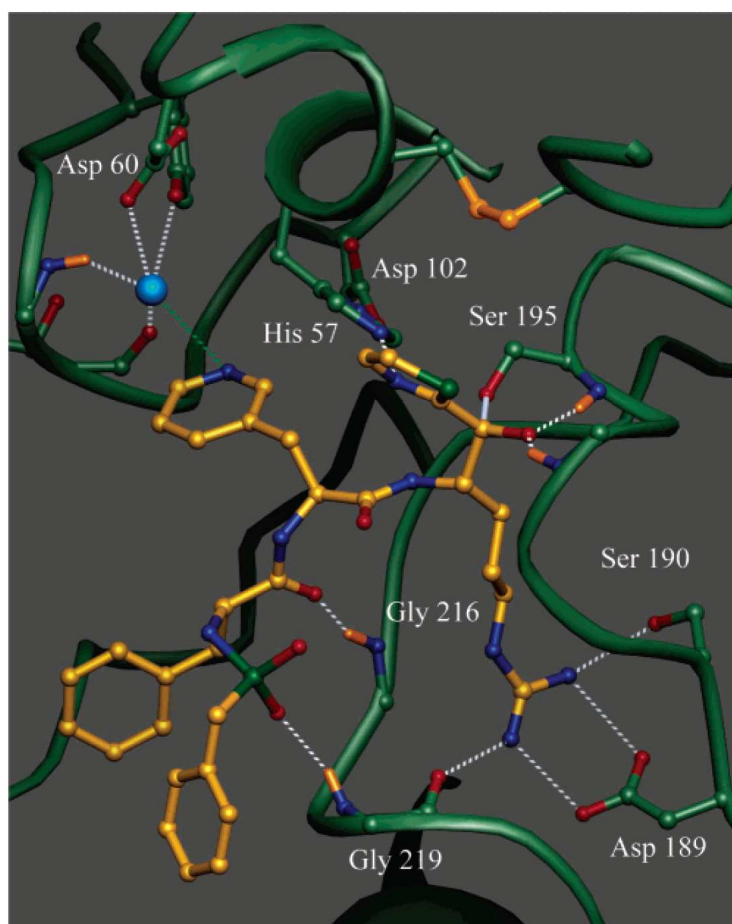
One of the surprises reported by Shiraishi *et al.* was the discovery of an increased selectivity against thrombin and FXa when increasing the size of P3, e.g. going from phenylalanine to biphenyl increased selectivity significantly.<sup>130</sup> From X-ray crystal structures it was found that only FVIIa is capable of accepting large P3 moieties, as a result of ligand-induced conformational changes of the 170-loop. This has not been observed previously. Even though FVIIa has an apparently smaller S3 than thrombin and FXa, this binding pocket surprisingly accepts larger groups than other serine proteases due to conformational changes.<sup>130</sup>

Only a few reports exist on the design and synthesis of peptidyl FVIIa inhibitors. Parlow and co-workers have pioneered the work on developing inhibitors for FVIIa and in addition they have been working on  $\alpha$ -ketothiazole peptidyl libraries using a D-Phe-AA-Arg scaffold.<sup>165,166</sup> This resulted in a range of low-micromolar FVIIa inhibitors proping S2 with different P2 substituents (see Figure 1.1.22).

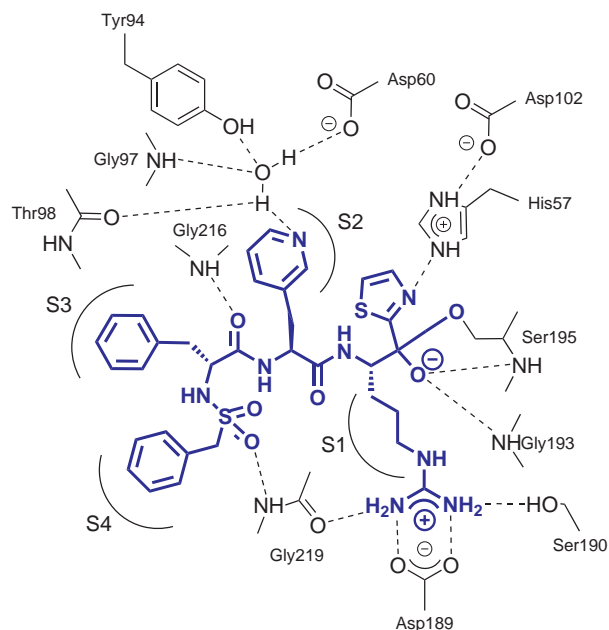


**Figure 1.1.22:**  $\alpha$ -Ketothiazole peptidyl FVIIa inhibitors proping the S2 binding site with a variety of different P2 substituents.<sup>165,166</sup>

Many compounds were synthesized probing S2 and the best one was **1.23** showing good potency for FVIIa and a selectivity factor of 500 against thrombin (see Figure 1.1.22). However, no selectivity at all was observed against FXa. Several carbonyl-reduced compounds were tested for biological activity, and as expected none of them inhibited FVIIa-TF.<sup>165,166</sup> An X-ray crystal structure of **1.23** revealed the binding mode of this inhibitor in the active site of FVIIa (see Figures 1.1.23 and 1.1.24).



**Figure 1.1.23:** Crystal structure of **1.23** bound in the active site of FVIIa-TF complex. Some of the key side chains of FVIIa are displayed (C: green, N: dark blue, O: red, S: yellow, H: orange). The carbon atoms of the inhibitor are shown in gold color. The hydrogen bonds formed by the inhibitor and a bound water molecule in the S2 binding pocket are shown in dotted white lines. The close interaction between the bound solvent and the pyridyl nitrogen is shown in the dotted green line.<sup>166</sup>



**Figure 1.1.24:** Binding mode of **1.23** determined by X-ray crystallography of FVIIa complexed with the inhibitor.<sup>165,166</sup>

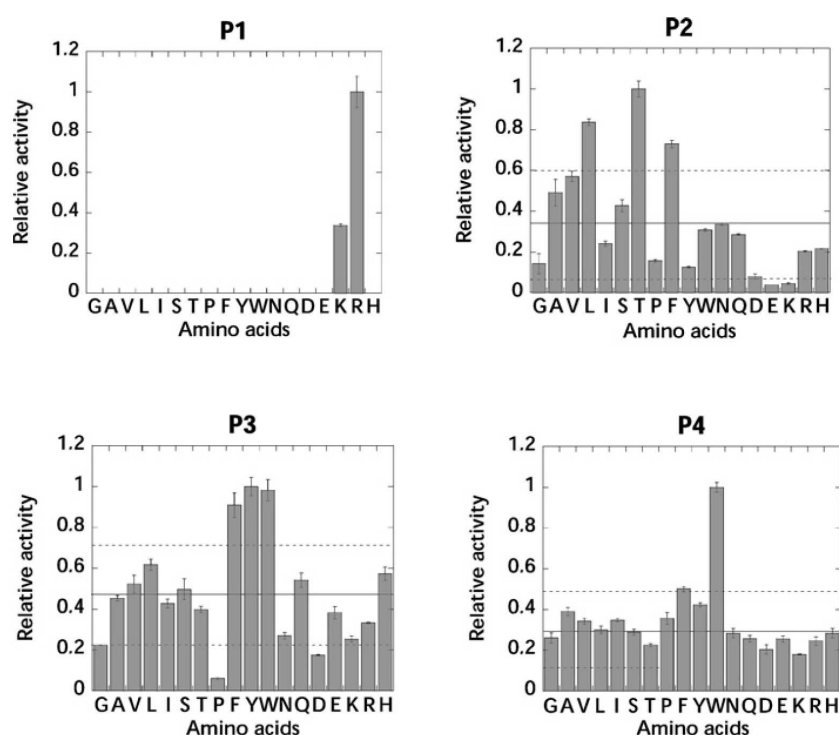
The P1 arginine residue forms the usual salt bridge with Asp189. The Ser195 side-chain forms a covalent bond to the activated carbon of the inhibitor.<sup>165,166</sup> The resulting oxyanion is stabilized in the oxyanion hole by the backbones of Ser195 and Gly193. Moreover, P1 forms two additional hydrogen bonds with the backbone of Gly219 and the Ser190. Ser190 is a unique feature of the S1 binding pocket of FVIIa, versus Ala190 in thrombin and FXa.<sup>128</sup> The thiazole ring interacts with the side-chain of His57. Binding in the S2 pocket with the unique Asp60 is achieved through multiple hydrogen bonds with water, whereas no specific bindings in the S3 pocket were observed.<sup>165,166</sup> The sulfonyl *N*-terminal forms a hydrogen bond with Gly219, which is located deep in the subsite of S1. From these results it seems that the benzyl sulfonyl group is occupying the S3 pocket rather than the S4 pocket. However, no specific binding with this group was observed from the X-ray crystal structure of FVIIa-TF complexed with **1.23**.<sup>165,166</sup>

### 1.1.4 Activity-based inhibitor design

A design and rational development of selective peptidyl protease inhibitors can be established using activity-based methods based on positional scanning, synthetic combinatorial libraries (PS-SCL).<sup>167</sup> The primary function of proteases is to discriminate among the many possible substrates available to it and to cleave specific proteins or peptides. The



understanding of the specificity and identification of the substates of the enzyme is therefore of great importance, which might also be used for design of protease inhibitors.<sup>167</sup> Since each binding pocket imparts its own specificity for an amino acid, the PS-SCL activity-based approach is to screen a protease against a synthetic combinatorial library composed of small molecules, in a format that systematically probes each binding pocket and collectively generates a positional profile based on ACC-linked peptides.<sup>167</sup>



**Figure 1.1.25:** The specificity profile of FVIIa-TF. In each library, the apparent second-order rate constant of the substrate hydrolysis were normalized to the sublibrary with the highest activity. Single-letter amino acid notation is used.<sup>168</sup>

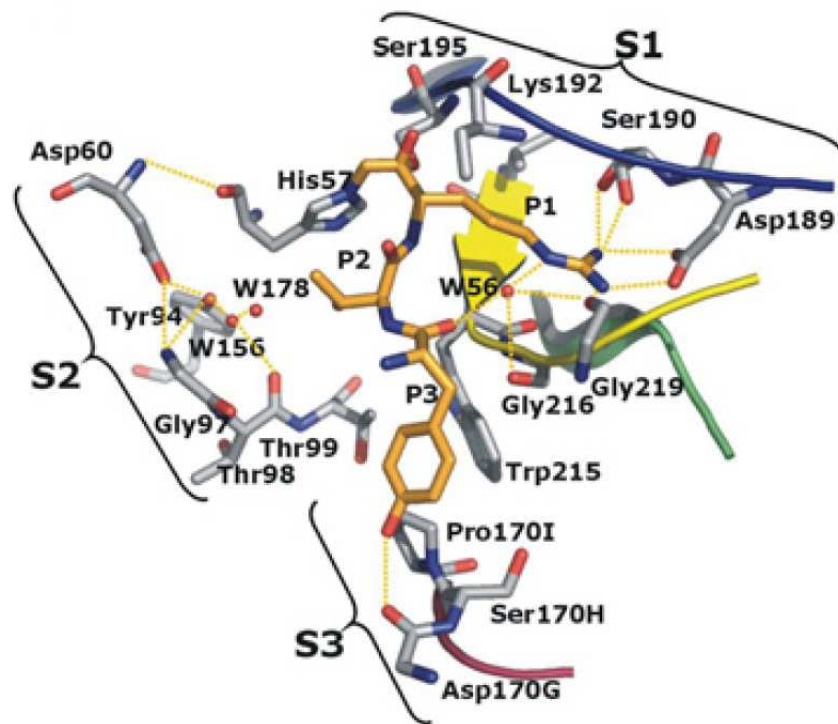
The typical PS-SCL is targeted against the non-prime side of the protease and is designed as a series of diverse sublibraries where one amino acid in a tetrapeptide is fixed and the remaining positions are a randomized mixture of amino acids.<sup>167</sup> One aspect that such libraries do not always fully address is the potential interdependence between the different binding pockets. Only one substrate-binding pocket is profiled at a time and therefore no steric considerations or interdependency between the pockets are investigated.<sup>168</sup>

PS-SCL has been used for design of a novel hexapeptide diphenylphosphonate inhibitor of Kaposi's sarcoma-associated herpes virus protease,<sup>169,170</sup> but never for designing of a specific serine protease inhibitor. However, a PS-SCL specificity profiling has been reported for FVIIa by researchers at Novo Nordisk (see Figure 1.1.25).<sup>168</sup> As expected, FVIIa only accepts arginine and lysine residues in the S1 pocket, with arginine being favored three-fold over lysine.<sup>168</sup> In the S2 pocket,  $\beta$ -branched or hydrophobic amino acids were allowed, with threonine > leucine > phenylalanine > valine being the preferred residues, whereas in the P3 position, large aromatic residues such as Phe, tyrosine and tryptophan were preferred.

The S4 pocket appeared to be highly promiscuous, although a slight preference for tryptophan was detected (see Figure 1.1.25). An identical specificity profile of FVIIa was obtained in the absence of TF, indicating that the cofactor did not alter the substrate specificity of FVIIa.<sup>168</sup> The most specific sequence was determined to be Trp-Tyr-Thr-Arg, which was subsequently crystallized with rFVIIa-TF as H-Trp-Tyr-Thr-Arg-cmk (see Figure 1.1.26). Of particular interest is the binding mode in S2 where the threonine residue was positioned adjunct to the catalytic triad with a distance of 3.4 Å to His57. This was the closest contact to any residue in the S2 pocket. Furthermore, the residue was within hydrogen-bonding distance with two water molecules and could be part of a hydrogen-bonding network of the S2 pocket involving the *para*-positioned hydroxy group of Tyr94, the carboxylate group of Asp60, the carbonyl of Thr98 and amides of both Gly97 and Thr98.<sup>168</sup> The P3 tyrosine residue interacted with the 170-loop including both a hydrogen bond and with hydrophobic interactions. The carbonyl group of the tyrosine residue hydrogen bonds with the amide group of Gly216 as well as with a water molecule. Thus, the preference for tyrosine, even compared with phenylalanine and tryptophan, is reflected by the extra hydrogen-bond donor of the side-chain, which leads to the more stabilized pocket as observed for the 170-loop.<sup>168</sup>

From modeling studies, the P4 tryptophan was expected to make favorable  $\pi$ - $\pi$  interactions with Trp215 in the bottom of the putative S4 pocket. However, the residue had no electron density corresponding to the P4 tryptophan, indicating that there is not a well-defined S4 binding pocket in FVIIa, despite the clear preference observed within the PS-SCL's.<sup>168</sup> This is to some extent supported by the structure as well as the fact that the natural substrate for FVIIa is the Asn\*-Leu-Thr-Arg↓Ile-Val-Gly-Gly site of FX (\* indicates that the amino acid is glycosylated amino acid).<sup>168</sup> The specificity profile of FVIIa clearly shows that this enzyme is not a very discriminative protease with a low degree of active-site specificity.

To elucidate any interdependency between the S4–S1 binding pockets, FVIIa was assayed with a total of 23 different ACC-linked tetrapeptides having arginine in P1, threonine or leucine in P2, tryptophan, tyrosine, leucine or alanine in P3 and tryptophan, phenylala-

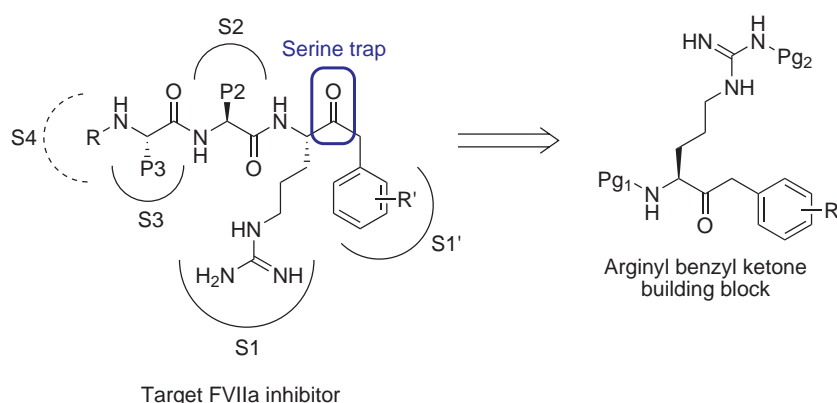


**Figure 1.1.26:** Crystal structure of FVIIa-sTF and Trp-Tyr-Thr-Arg-cmk obtained at a 2.05 Å resolution. Specific interactions between the selected active-site residues and the covalently bound inhibitor are highlighted. The S1, S2 and S3 compositions are shown with brackets, and residue identification are with chymotrypsin numbering.<sup>168</sup>

nine or asparagine in P4.<sup>168</sup> These substrates were selected partly to address different levels of activity based on the specificity profile and partly to explore the space around the natural FX cleavage site Asn\*-Leu-Thr-Arg↓Ile-Val-Gly-Gly.<sup>168</sup> It was found, that S3 selectivity is probably driven or influenced by P4 occupancy, because P4 substitution of asparagine to tryptophan or phenylalanine is very different for substrates with different P3 residues, whereas minor differences were observed between similar substrates with leucine or threonine in P2. The optimal binding pocket occupancy does therefore not translate into one optimal substrate.

## 1.2 Aim of the thesis

The aim of this PhD thesis (Chapter 1–4) is to synthesize a range of reversible FVIIa inhibitors. The ultimate inhibitor is water soluble with high selectivity against FVIIa and with medium to high potency (low micromolar to nanomolar). It should be non-toxic *in vivo*, possess a high plasma clearance and finally be able to stabilize rFVIIa in a liquid formulation of NovoSeven<sup>®</sup> for storage at room temperature for at least 36 months.



**Figure 1.2.1:** The target peptidyl FVIIa inhibitors consists of a tripeptide sequence with a benzyl ketone serine trap, which potentially also probes the S1' pocket. Such inhibitors are projected to be synthesized from the arginyl benzyl ketone building block shown to the right.

A peptidyl inhibitor scaffold was chosen (see Figure 1.2.1), partly because sequence alternation is much easier done to explore the SAR, partly because a peptide much better resembles the natural substrate of FVIIa and finally because peptides usually show lower toxicity than small molecules as they degrade into natural building blocks. Bioavailability is not a concern for those target molecules. Ideally, they should only inhibit FVIIa in the formulation and not in the body. Synthesis of novel peptidyl FVIIa inhibitors is furthermore of great interest due to the almost absence of this class of selective serine protease inhibitors in the literature.

Design of reversible and selective FVIIa inhibitors is based on two strategies; first of all, introduction of an electrophilic functionality (serine trap) in the P1–P1' position (C-terminal). Many serine traps have been reported for thrombin and other serine proteases, but the benzyl ketone C-terminal functionality is almost completely unexplored for any of these proteases. As well as the interesting exploration of this unknown serine trap, it could be a way for the introduction of functional groups probing the S1' binding pocket of FVIIa. Favorable interactions of the P1' residue might induce increased selectivity and

potency. Secondly, selection of the peptide sequence will be based on a specificity profile for FVIIa.<sup>168</sup> Such a strategy has not previously been utilized for design of selective blood coagulation serine inhibitors. Based on the FVIIa specificity profile a tripeptide sequence will be considered, because the S4 binding pocket seems to be rather undefined.<sup>168</sup> An arginine residue has to be chosen at the P1 position exclusively, because the S1 binding pocket has a 3-fold selectivity for arginine over lysine, but does not accept any other amino acid residue there.<sup>168</sup> Finally, different *N*-terminals will be explored inspired by the literature, which has demonstrated some interactions with subsites of S1 or interactions reaching the putative S4 binding pocket.

For appropriate functionalization of the arginyl benzyl ketone C-terminal building block, two new synthetic methodologies will be investigated utilizing mild reaction conditions (see Chapter 2). The target building block synthesis is projected to employ cheap and commercially available starting materials. Furthermore, all transformations should be easy and robust, preferable with catalytic rather than stoichiometric reagents not leading to any significant racemization.

When an appropriate building block synthesis is established a library consisting of the novel peptidyl benzyl ketone inhibitors will be synthesized (see Chapter 3). To elucidate the potency and selectivity of the peptides, several *in vitro* competitive binding assays will be run for a variety of blood coagulation serine proteases. Based on potency, selectivity and solubility in aqueous media, the peptides will be evaluated for potential usage as a stabilization agent for liquid formulations of FVIIa. However, development of new FVIIa formulations as well as long-term studies of the enzyme stability are beyond the scope of this project.



## Chapter 2

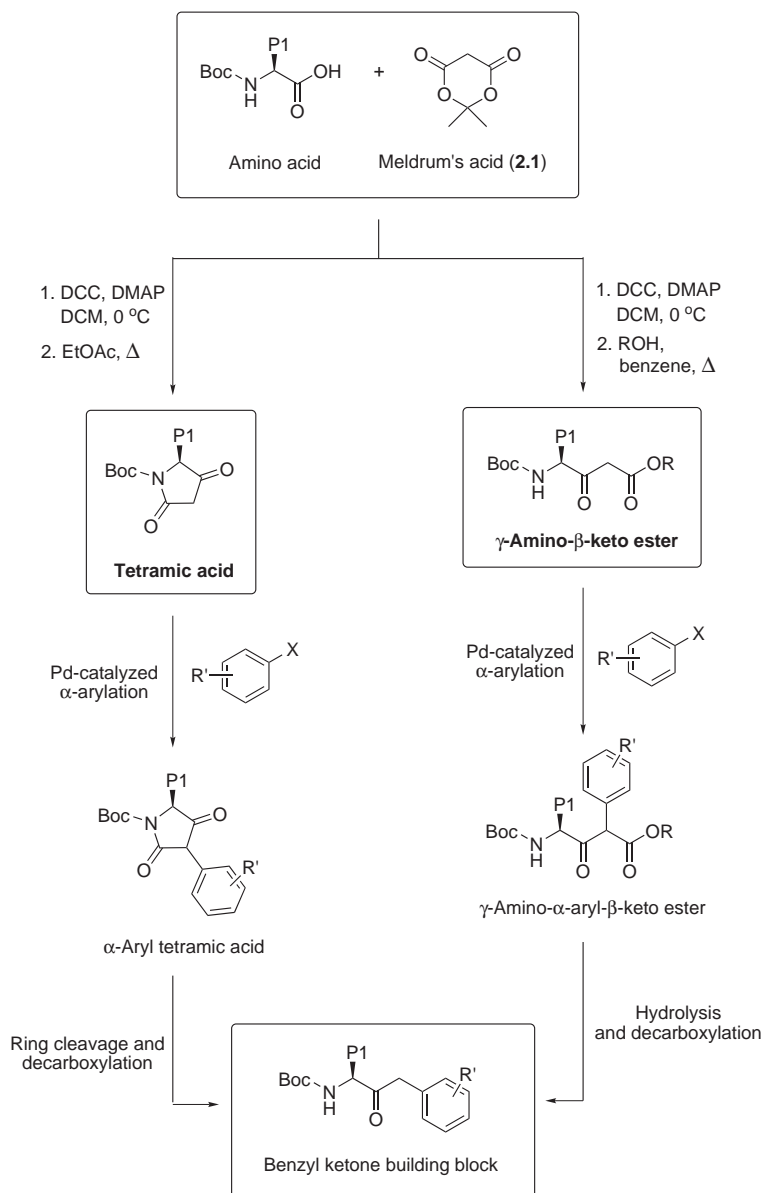
# Building block synthesis via palladium-catalyzed $\alpha$ -arylation

The aim of this chapter of the thesis is to describe novel methods toward the synthesis of the arginyl benzyl ketone building block. These methods should utilize mild reaction conditions providing a high functional group tolerance, they should be easy to perform and use commercially available starting materials only. These improvements would first of all allow the introduction of a variety of functional groups at the benzyl group for probing the S1' pocket of FVIIa. Such an approach is almost absent in the literature and would provide valuable information about the FVIIa pharmacophore and potentially improve selectivity and potency of the synthesized inhibitors. Furthermore, development of mild reactions would be an advantage for industrial scale-up and production, which is of great relevance for Novo Nordisk.

### 2.1 Synthetic strategies

Two strategies are proposed here involving two different key intermediates; a  $\gamma$ -amino- $\beta$ -keto ester and a tetramic acid, respectively (see Scheme 2.1.1). These two intermediates can both be synthesized by *C*-acylation of Meldrum's acid (**2.1**),<sup>171-174</sup> which represents a remarkable, mild method for *C-C* bond formation.

Both strategies involve, in the second step, an  $\alpha$ -arylation, which requires the selection of suitable conditions among different coupling partners, catalysts, ligands and solvents. This transformation will afford a  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto ester or an  $\alpha$ -aryl tetramic acid, respectively. In fact palladium-catalyzed  $\alpha$ -arylation reactions are often performed in mild conditions with a high functional group tolerance. Moreover, the reactions are run with catalytic conditions, which is both convenient for the subsequent purification and environmental economically. The last step of both strategies is an ester/lactam hydrolysis



**Scheme 2.1.1:** Synthetic strategies toward the benzyl ketone building block through either a  $\gamma$ -amino- $\beta$ -keto ester or a tetramic acid.

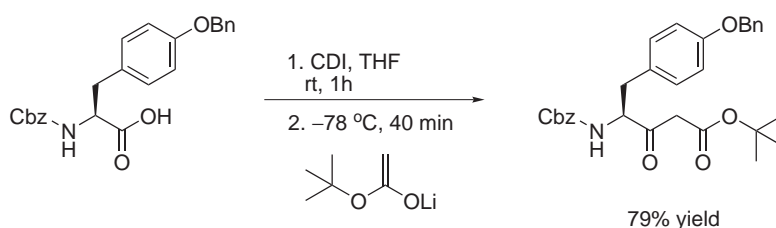
followed by a decarboxylation which would lead to the benzyl ketone building block in both cases (see Figure 2.1.1). Phenylalanine will be used for reaction development, but ultimately the chemistry should be applied to arginine (P1) with an appropriate N<sup>ω</sup>-protection for the subsequent peptide synthesis.



## 2.1.1 $\gamma$ -Amino- $\beta$ -keto esters

### 2.1.1.1 Traditional syntheses

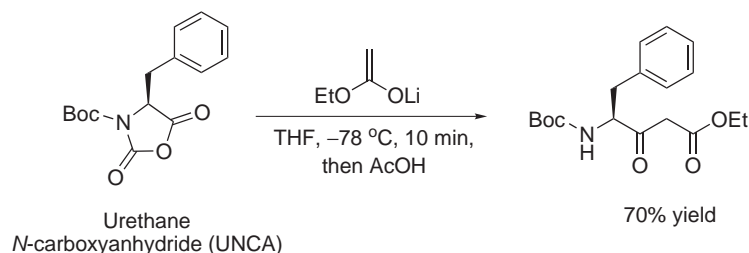
$\gamma$ -Amino- $\beta$ -keto esters are a group of important compounds for the synthesis of e.g. the corresponding  $\beta$ -hydroxy acids<sup>175</sup> which are found in many natural products. Traditionally  $\gamma$ -amino- $\beta$ -keto esters are synthesized by cross-Claisen condensation with lithium enolates typically with a CDI-activation. This method has been employed for the synthesis of (3*S*,4*R*)-statines,<sup>176,177</sup> total synthesis of the cyclodepsipeptides didemnins A, B and C,<sup>178</sup> ketomethylene and hydroxymethylene peptide isosters<sup>179–181</sup> in modest to good yield (see Scheme 2.1.2 for an example). Magnesium enolates have also been applied affording good yields.<sup>182–185</sup> C-Acylation is favored over O-acylation when magnesium enolates are used. These reagents are almost neutral which provide a better functional group tolerance than lithium enolates,<sup>186</sup> but the use of organometallic reagents in excess is always a concern especially regarding to functional group tolerance.



**Scheme 2.1.2:** Synthesis of Cbz-protected  $\gamma$ -amino- $\beta$ -keto tert-butyl ester by reaction with a lithium enolate.<sup>180</sup>

The CDI group has shown to be the most efficient activation group for the preparation of  $\beta$ -keto esters,<sup>176–180,187–190</sup> whereas other activations such as mixed anhydride, acid chlorides and Weinreb amides gave only moderate yields.<sup>191–193</sup> Unfortunately, CDI is rather expensive for industrial use. Optimizations of the cross-Claisen condensation have been reported, e.g. by using *N,N*-dibenzyl phenylalanine benzyl esters.<sup>194</sup>

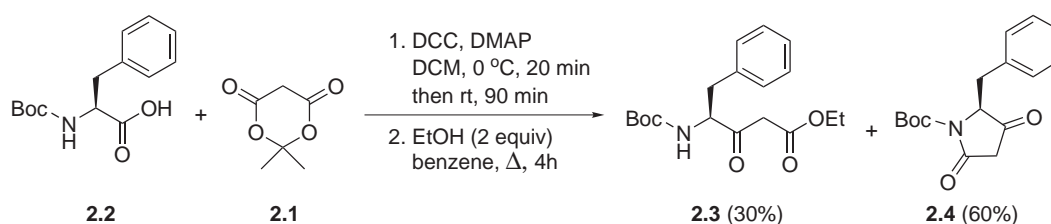
Besides of the cross-Claisen condensation described above,  $\gamma$ -amino- $\beta$ -keto esters can also be synthesized by treating urethane *N*-carboxyanhydrides (UNCAs) with lithium enolates<sup>23,195</sup> (see Scheme 2.1.3) or by ozonolysis and reductive work-up of cyclohexadiene derivatives.<sup>196</sup> Unfortunately, these methods require starting materials that are not readily available.



**Scheme 2.1.3:** Synthesis of a  $\gamma$ -amino- $\beta$ -keto ester by treatment of a urethane *N*-carboxyanhydride (UNCA) with lithium enolate of EtOAc.<sup>195</sup>

### 2.1.1.2 Facile synthesis using Meldrum's acid

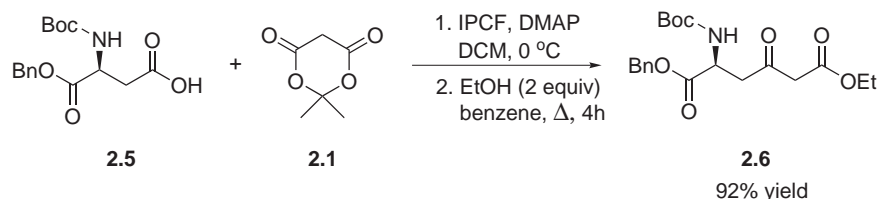
In 1999 Franck and co-workers<sup>174</sup> reported a facile synthesis of  $\gamma$ -amino- $\beta$ -keto esters by C-acylation of Meldrum's acid (**2.1**). Unfortunately, yields were very low (up to 30%) because of the competitive formation of the corresponding tetramic acid. For example, the  $\gamma$ -amino- $\beta$ -keto ester **2.3** was synthesized in 30% yield, giving in addition the tetramic acid **2.4** as the major (by)-product (see Scheme 2.1.4).



**Scheme 2.1.4:** Synthesis of the  $\gamma$ -amino- $\beta$ -keto ester **2.3** by C-acylation of Meldrum's acid (**2.1**) with Boc-Phe-OH (**2.2**). Unfortunately, the yield is very low (30%) due to formation of the tetramic acid (**2.4**).<sup>174</sup>

It was however noted, that *N*-phthaloylglycine gave exclusively the  $\gamma$ -amino- $\beta$ -keto ester because the amino group was fully protected.<sup>174,197</sup> Another successful application of this method was the preparation of  $\delta$ -amino- $\beta$ -keto esters. Protected aspartic acid **2.5** was converted to the ethyl ester **2.6** with Meldrum's acid (**2.1**) in 92% yield (see Scheme 2.1.5). This synthesis afforded no cyclization, presumably because 6-membered rings form less easily compared to 5-membered rings.<sup>174</sup>

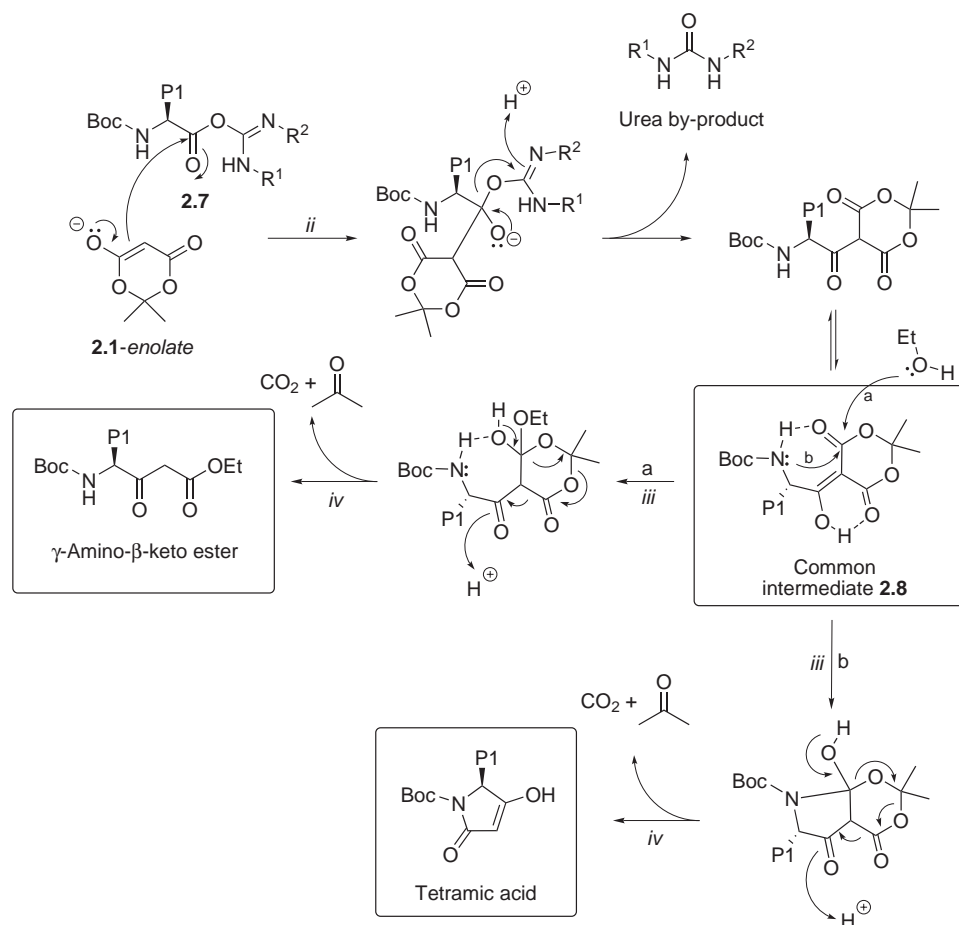
Usually, tetramic acids are synthesized analogously, but with refluxing EtOAc instead of EtOH/benzene. The explanation of formation of either the  $\gamma$ -amino- $\beta$ -keto ester or the tetramic acid may be found in the mechanism. The mechanism consists of four



**Scheme 2.1.5:** C-Acylation of Meldrum's acid (**2.1**) with protected aspartic acid **2.5** afforded the ethyl ester **2.6** in high yield. No cyclization was observed.<sup>174</sup>

steps;<sup>171</sup> *i*) activation of the amino acid, *ii*) C-acylation of Meldrum's acid (**2.1**),<sup>198</sup> *iii*) intramolecular attack from an alcohol or intermolecular attack from the amino group resulting in a cyclization, *iv*) elimination of CO<sub>2</sub> and acetone.

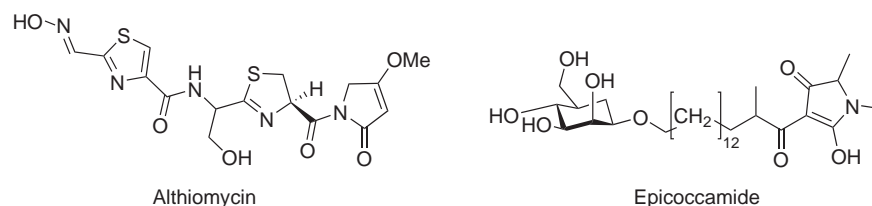
Figure 2.1.1 shows steps *ii-iv* of the mechanism, which can lead to both of the key intermediates depending on the work-up conditions of the common intermediate **2.8**. This intermediate is formed by activation of a *N*-Boc protected amino acid initially forming an *O*-acylisourea intermediate **2.7** (if a carbodiimide is used for activation), which is subsequently attacked by the enolate of Meldrum's acid (**2.1-enolate**) resulting in **2.8**. The two possible products, the  $\gamma$ -amino- $\beta$ -keto ester and the tetramic acid, are formed upon treatment with EtOH/benzene (path *a*) or EtOAc (path *b*), respectively. Formation of the tetramic acid is highly favored by hydrogen bonding facilitating an intermolecular attack.<sup>171</sup> These observations explain why the  $\gamma$ -amino- $\beta$ -keto ester is only formed in low yield with the tetramic acid as the major (by)-product, and why *N*-phthaloylglycine gave the corresponding  $\beta$ -keto ester exclusively. However, the literature does not contain any specific details why intermolecular attack is less favored in benzene and more favored in EtOAc.



**Figure 2.1.1:** Mechanism for the formation of a  $\gamma$ -amino- $\beta$ -keto ester or a tetramic acid, respectively, by C-acylation of Meldrum's acid (2.1). a: EtOH (2 equiv), benzene,  $\Delta$ . b: EtOAc,  $\Delta$ .

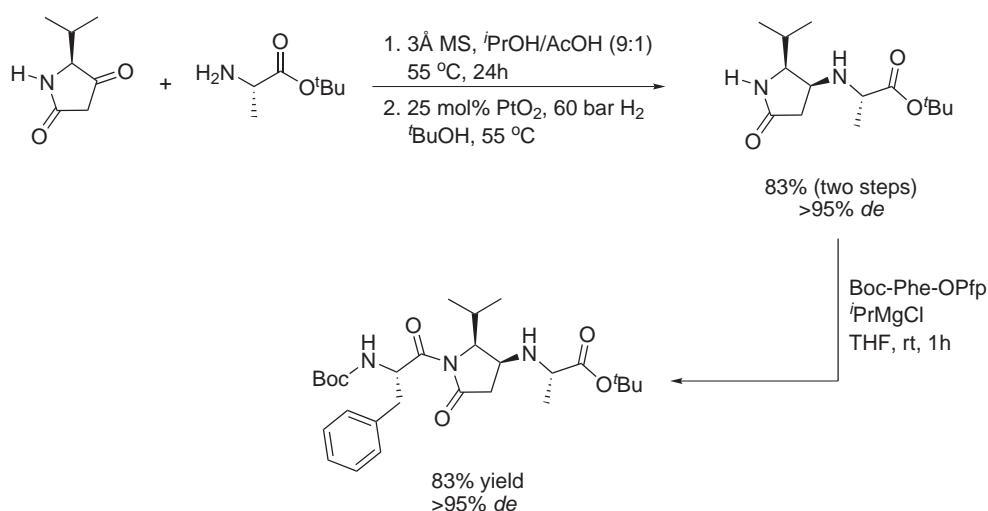
## 2.1.2 Tetramic acids

Tetramic acids are  $\beta$ -keto- $\gamma$ -lactams which are slightly acidic ( $pK_a \approx 6.4$ ).<sup>199,200</sup> Depending on solvent, concentration and temperature, tetramic acids can exist as both an enol and a keto tautomer, with the latter of the two known as 2,4-pyrrolidindiones.<sup>199,201–203</sup> The structural unit of tetramic acids has been known for more than 100 years,<sup>204,205</sup> and it is found in many biologically active natural products,<sup>201,202</sup> typically either as 3-acyl or 4-*O*-alkyl derivatives, examples being althiomycin,<sup>206,207</sup> dolastatin 15,<sup>208,209</sup> and epicoccamide<sup>210</sup> (see Figure 2.1.2).



**Figure 2.1.2:** *Althiomycin*<sup>206,207</sup> and *epicoccamide*<sup>210</sup> are two natural products containing the tetramic acid moiety.

Althiomycin, which is a *N*-acyl-4-methoxy-3-pyrrolin-2-one, was isolated from *Streptomyces althioticus* in 1957<sup>206</sup> and is a broad-spectrum antibiotic functioning by inhibition of the DNA translation.<sup>207</sup> So far, it is unknown which enantiomer is the active one, because purification of althiomycin results in racemization.<sup>201</sup> More recently in 2003, epicoccamide was isolated from the fungus *Epicoccum purpurascens*.<sup>210</sup> This natural product consists of three different substructures; a glycoside, a fatty acid and a tetramic acid (see Figure 2.1.2). However, the biological function of epicoccamide has not yet been found.

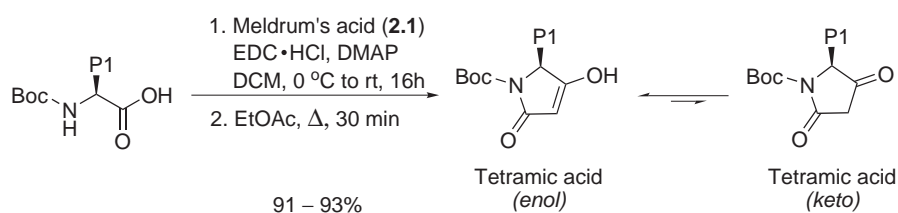


**Scheme 2.1.6:** Incorporation of tetramic acids into peptides which increased the *in vitro* stability compared to the parent tripeptide.<sup>211,212</sup>

Tetramic acids are furthermore important intermediates in the synthesis of statins,<sup>171,213</sup>  $\gamma$ -amino- $\beta$ -hydroxy acids<sup>214</sup> and lactam renin inhibitors<sup>215</sup> Moreover, tetramic acid derivatives have been reported as key intermediates for the synthesis of analogs of penicillins

and cephalosporins<sup>216</sup> and 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives<sup>217</sup> which are inhibitors of glycolic acid oxidase. 2-Ethyl-4,6-dimethylphenyl-substituted tetramic acid derivatives have been described in the patent literature as novel pesticides and herbicides.<sup>218</sup> Recently, methods for incorporation of amino acid-derived tetramic acids into peptides have been developed,<sup>211,212,219</sup> giving rise to more stable tripeptides *in vitro* (see Scheme 2.1.6).

Previously, tetramic acids were synthesized by base-catalyzed Dieckmann-cyclizations,<sup>199,201</sup> which led to 10–30% racemization<sup>220</sup> and formation of an anhydrodimer.<sup>199</sup> The method therefore is not very convenient and requires several synthetic steps. In 1987, P. Jouin *et al.*<sup>171</sup> developed a synthesis of amino acid-derived tetramic acids giving no racemization. Later on, several groups reported the use of carbodiimides (e.g. DCC<sup>172,173</sup> and EDC<sup>211,212,219</sup>) as coupling agents instead of the originally reported IPCF (see Scheme 2.1.7).

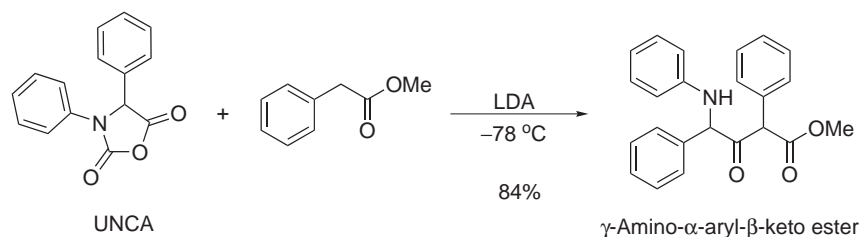


**Scheme 2.1.7:** Synthesis of amino acid-derived tetramic acids from a *N*-Boc-protected amino acid and Meldrum's acid (2.1).<sup>219</sup>

Carbodiimides are excellent activation agents and are traditionally used for peptide synthesis.<sup>221</sup> They are robust and do not require anhydrous reaction conditions like acid chlorides, anhydrides or IPCF.<sup>171,221</sup> The urea derivative by-product from DCC may be difficult to remove when using solution phase synthesis,<sup>222</sup> therefore the acid soluble EDC is a better choice for solution-phase peptide couplings.<sup>211,212,219,223</sup>

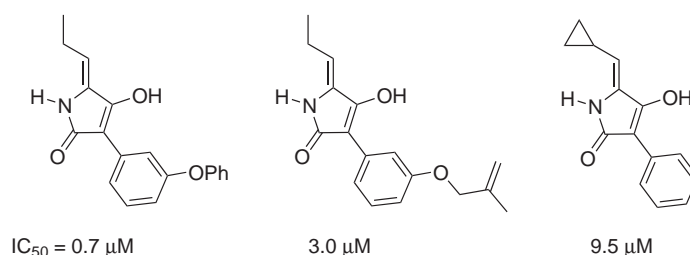
### 2.1.3 Palladium-catalyzed $\alpha$ -arylations

No general procedures exist in the literature for preparation of  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto esters or  $\alpha$ -aryl tetramic acids. However, both classes of compounds have been described. For example, Palomo and Cossio<sup>23</sup> has reported the synthesis of a  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto ester synthesized from an UNCA by reaction with methyl 2-phenylacetate (see Scheme 2.1.8).



**Scheme 2.1.8:** One of the only reports describing the synthesis of an  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto ester is the reaction of an UNCA with methyl 2-phenylacetate upon treatment with LDA.<sup>23</sup>

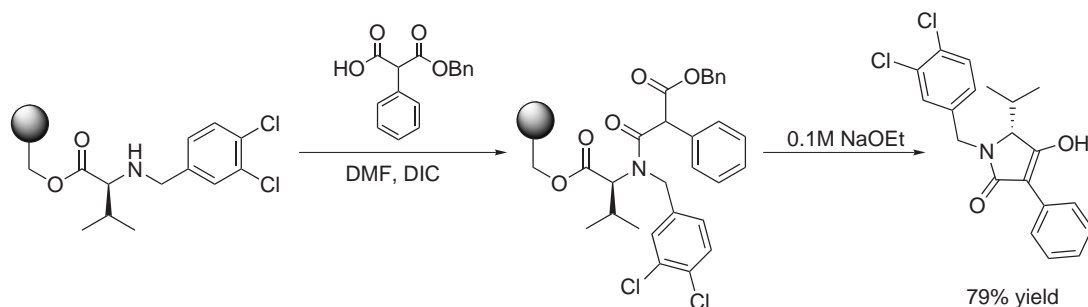
$\alpha$ -Aryl tetramic acids are also only briefly described in the literature,<sup>224,225</sup> the most important being 3-phenyl 5-olefinic tetramic acids as novel glycine site *N*-methyl-D-aspartate receptor antagonists for the treatment of neurological diseases (see Figure 2.1.3).<sup>226</sup>



**Figure 2.1.3:** 3-Phenyl 5-olefinic tetramic acid show inhibition of the binding of [ $\beta$ H]-L-689,560 to the strychnine insensitive glycine site on the rat brain membranes down to  $\text{IC}_{50} = 0.7\text{ }\mu\text{M}$ .<sup>226</sup>

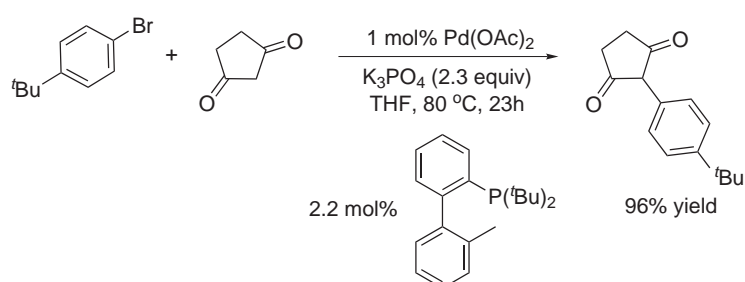
The development of a solid-phase synthesis of substituted  $\alpha$ -aryl tetramic acids has also been described<sup>227</sup> (see Scheme 2.1.9). However, none of these methods utilize the easy synthesis of *N*-Boc protected tetramic acids described above, and the methods are not general, requiring the use of strong base (e.g. LDA or NaOEt), consist of several synthetic steps and use non-general *N*-protection groups. These methods do not represent an efficient synthesis for a broad range of  $\alpha$ -aryl substituted amino acid-derived tetramic acids.

Traditionally,  $\alpha$ -arylated ketones or carboxylic acid derivatives have been synthesized by nucleophilic aromatic substitution reactions ( $\text{S}_{\text{N}}\text{Ar}$ ) of aryls substituted with electron-withdrawing groups by reaction with stabilized enolates<sup>228</sup> or via copper-catalyzed eno-



**Scheme 2.1.9:** Solid-supported synthesis of  $\alpha$ -aryl tetramic acids by reaction with a 2-phenylpropanoic acid derivative.<sup>227</sup>

late reactions with 2-bromobenzoic acid.<sup>229</sup> These methods all have drawbacks and are not very general. Usually, they require harsh reaction conditions, which are not suitable for protected, enantiomerically pure amino acid derivatives. Using a palladium-catalyzed  $\alpha$ -arylation would be much more efficient since these reactions are typically more general, mild and broad in substrate scope.



**Scheme 2.1.10:**  $\alpha$ -Arylation of cyclopentane-1,3-dione with an aryl bromide in the presence of 1 mol%  $\text{Pd}(\text{OAc})_2$  and 2.2 mol% *t*Bu-MePhos ligand.<sup>230</sup>

The literature reports a number of palladium catalyzed  $\alpha$ -arylation conditions for different substrates containing electron-withdrawing groups such as ketones,<sup>230</sup> aldehydes,<sup>231</sup> malonates,<sup>232</sup> cyanoesters,<sup>232</sup> sulfones,<sup>233</sup> esters,<sup>234</sup> amides,<sup>235</sup> protected amino acids,<sup>236</sup> piperidinones<sup>237</sup> and nitriles.<sup>238</sup> Only few examples of  $\alpha$ -arylation of 1,3-dicarbonyls have been described, and most of them are non-chiral and synthetically simple compounds. Most of the substrates are reported by Buchwald<sup>230</sup> and Hartwig,<sup>232</sup> using substrates such as diethyl malonate, 1,3-cyclohexanedione, 1,3-cyclopentanedione and amino acids (see Scheme 2.1.10 for an example).  $\alpha$ -Arylation of  $\gamma$ -amino- $\beta$ -keto esters has not been reported in the literature, but Buchwald and co-workers<sup>230</sup> have arylated diethyl malonate with 1-bromo-4-*tert*-butylbenzene under almost the same conditions as



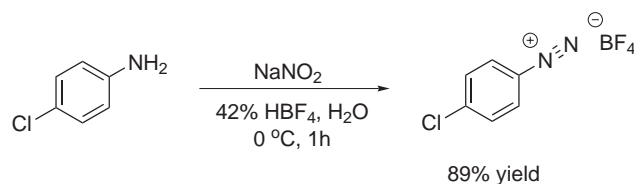
showed on Scheme 2.1.10 (70 °C, 10h, 92% yield). Very recently more functionalized substrates have been subjected to palladium-catalyzed arylations, e.g. the  $sp^2$  arylation of azine *N*-oxides,<sup>239</sup>  $\alpha$ -arylation of highly functionalized cyclohexanones,<sup>240</sup> and asymmetric intramolecular  $\alpha$ -arylation of aldehydes.<sup>241</sup>

Even though no general reaction conditions exist in the literature, it is possible to discern a general trend for the reaction of substrates similar to  $\beta$ -keto esters and tetramic acids. It was found that an  $\alpha$ -arylation is usually conducted with either  $\text{Pd}(\text{OAc})_2$  or  $\text{Pd}_2(\text{dba})_3$  using an aryl bromide or iodide.<sup>230–238</sup> Aryl chlorides are often too unreactive for this type of chemistry. Many different solvents can be used, but THF, toluene, dioxane, MeCN or DMF are the most common. The bases used can be divided into two groups; weak inorganic bases such as  $\text{Cs}_2\text{CO}_3$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{K}_3\text{PO}_4$ ,  $\text{Na}_3\text{PO}_4$  or  $\text{Na}_2\text{CO}_3$  and strong organic bases such as  $\text{NaO}^t\text{Bu}$ , KHMDS, NaHMDS, LDA or  $\text{LiN}(\text{SiMe}_2\text{Ph})_2$ . The choice of base is strongly dependent on the  $\text{p}K_a$  value of the substrate but, in general, strong bases can be used for most simple substrates. However, if base sensitive functionalities are present in the molecule or deprotonation can cause racemization, strong bases may give problems. Sometimes strong bases even require a two-step procedure with addition of base at reduced temperature and then the actual arylation at elevated temperature.<sup>237</sup> Buchwald and co-workers<sup>230</sup> reported the first use of a weak inorganic base ( $\text{K}_3\text{PO}_4$ ) in palladium catalyzed  $\alpha$ -arylations of ketones. Finally, a very important parameter is the choice of ligand. An appropriate ligand can be difficult to find, since many different ligands have been reported to work in  $\alpha$ -arylation of carbonyl substrates, with a broad variety of stereo- and electronic properties. Thus, in general the ligands are either mono- or bis-phosphines, such as  $\text{P}(^t\text{Bu})_3$ , BINAP, Xantphos and biaryl phosphines.<sup>230</sup>

### 2.1.3.1 Utilization of aryl diazonium salts

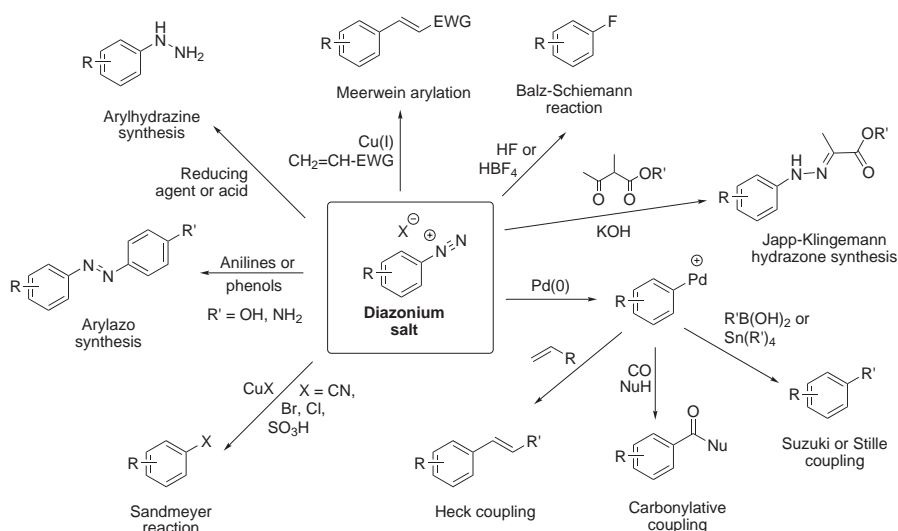
In the literature, several reports on palladium-catalyzed cross-couplings utilizing aryl diazonium salts have been published. Diazonium salts are synthesized from the corresponding anilines by treatment with  $\text{NaNO}_2$  in the presence of an acid, e.g.  $\text{HCl}$ ,<sup>242</sup>  $\text{HBr}$ ,<sup>243</sup>  $\text{H}_2\text{SO}_4$ ,<sup>244</sup>  $\text{HPF}_6$ <sup>245</sup> or  $\text{HBF}_4$ <sup>246–248</sup> determining the diazonium salt counterion (see Scheme 2.1.11 for an example). Most of the diazonium salts are considerably unstable resulting in elimination of nitrogen (which is an excellent leaving group)<sup>249,250</sup> and thus they are potentially explosive. However, salts featuring low nucleophilic counterions, e.g. tetrafluoroborates or hexafluorophosphates, can be isolated and manipulated and many of them are commercially available.<sup>251</sup> One-pot procedures forming the diazonium salt *in situ* is a convenient alternative to procedures where it has to be isolated.<sup>252</sup>

The advantages of using diazonium salts as coupling partners are economically because anilines are cheaper than the corresponding aryl halides and it furthermore broadens the



**Scheme 2.1.11:** Aryldiazonium tetrafluoroborates are synthesized from the corresponding anilines by treatment with  $\text{NaNO}_2$  in aqueous  $\text{HBF}_4$ .<sup>246–248</sup>

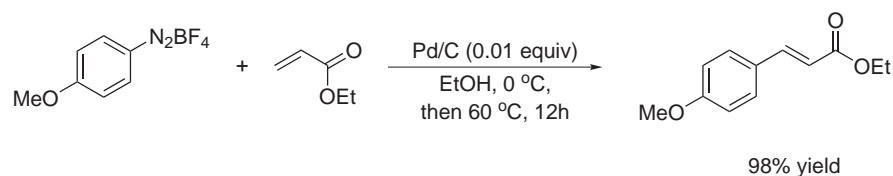
substrate scope. Utilization of aryldiazonium salts might also open the possibility of using remarkably mild reaction conditions ( $\text{MeCN}$ ,  $\text{H}_2\text{O}$ , no ligand or base, rt), cheaper catalysts ( $\text{PdCl}_2$  or  $\text{Pd/C}$ ) and allow for synthesis of *ortho*-substituted  $\alpha$ -aryl tetramic acids, since such conditions are often used in connection with aryldiazonium salts.<sup>253–255</sup>



**Figure 2.1.4:** Aryldiazonium salts are involved in many other reactions besides of palladium-catalyzed cross-couplings making them useful intermediates in synthetic chemistry.<sup>253</sup>

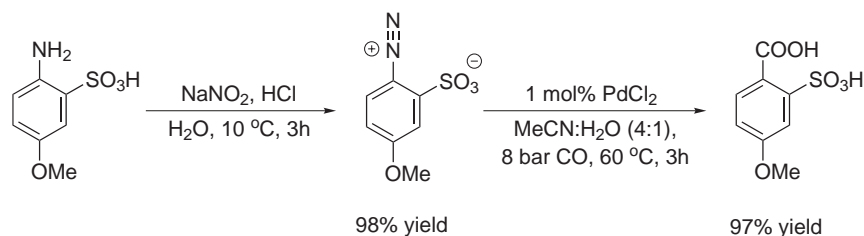
Many examples exist in the literature illustrating the broad utilization of aryldiazonium salts in palladium-catalyzed reactions, such as Heck,<sup>255–257</sup> Suzuki,<sup>258,259</sup> Stille<sup>260</sup> and carbonylative<sup>261–263</sup> cross-couplings (see Figure 2.1.4). Moreover, aryldiazonium salts can participate in a variety of non-palladium-catalyzed reactions, such as the Meerwein,<sup>264</sup> Sandmeyer,<sup>265</sup> Balz-Schiemann<sup>266</sup> and Japp-Klingemann<sup>267</sup> reaction as well as reactions forming arylhydrazines<sup>268,269</sup> and arylazo compounds.<sup>270</sup>

In the literature, many interesting examples exist of palladium-catalyzed cross-couplings performed under remarkable mild conditions. For example, Beller and co-workers<sup>255</sup> described a Heck cross-coupling catalyzed by Pd/C (see Scheme 2.1.12).



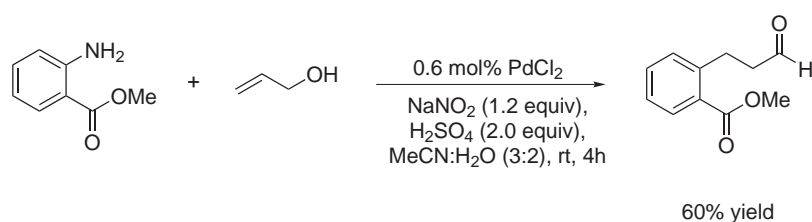
**Scheme 2.1.12:** Heck cross-coupling using an aryldiazonium salt catalyzed by Pd/C in excellent yield (many examples).<sup>255</sup>

A palladium-catalyzed carboxylation of an aryldiazonium inner salt catalyzed by 1 mol% PdCl<sub>2</sub> was reported by Siegrist and co-workers (see Scheme 2.1.13).<sup>254</sup> This afforded 2-sulfo-4-methoxybenzoic acid in high yield, which is a key intermediate for the synthesis of a herbicide. The synthesis can be carried out as a two-step procedure as shown or as a one-pot reaction with no isolation of the aryldiazonium salt, which would give considerably cost savings on industrial scale.<sup>254</sup>



**Scheme 2.1.13:** Synthesis of 2-sulfo-4-methoxybenzoic acid in high yield from the aryldiazonium salt through a palladium-catalyzed carboxylation.<sup>254</sup>

One of the major problems with *in situ*-generated aryl diazonium salts is the oxidizing character of  $\text{NaNO}_2$ , which could interfere with the  $\text{Pd}(0)$  species assumed to insert into the  $\text{C}-\text{N}_2^+$  bond via an oxidative addition.<sup>254</sup> For this reason, the excess of nitrite could be destroyed after the diazotization, e.g. with sulfamic acid ( $\text{H}_3\text{NSO}_3$ ).<sup>254</sup> However, this is not always the case; Zaragoza has reported a one-pot diazotization and Heck coupling for the synthesis of 3-arylpropanals (see Scheme 2.1.14).<sup>271</sup>



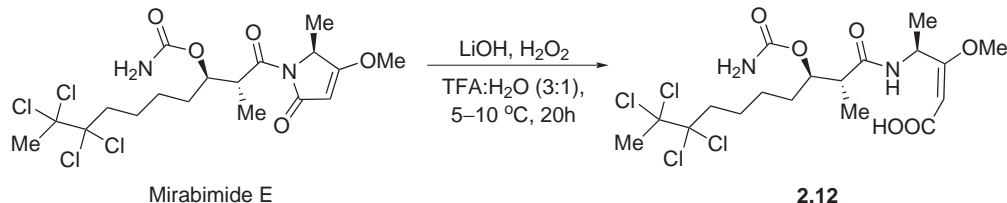
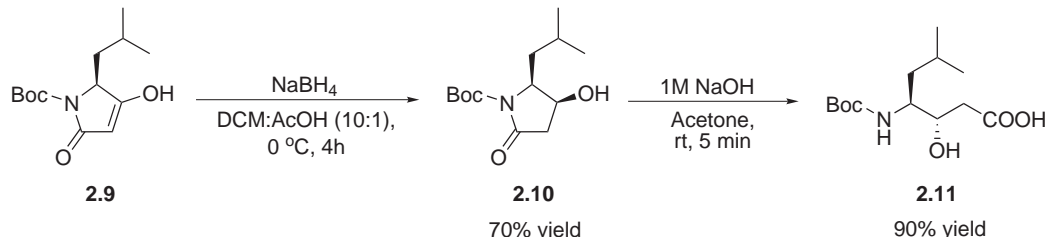
**Scheme 2.1.14:** One-pot diazotization and Heck coupling of methyl anthranilate affording a 3-arylpropanal in good yield.<sup>271</sup>

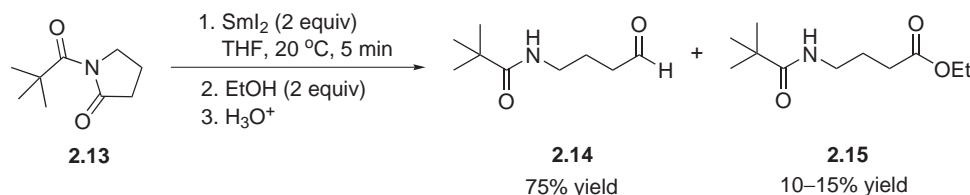
This reaction is unusual in many ways; it takes place in  $\text{MeCN}$  and  $\text{H}_2\text{O}$  and thus anhydrous solvents are not required, neither is an inert atmosphere and the reaction does not have to be heated or cooled. Only a catalyst loading of 0.5–1.0 mol%  $\text{PdCl}_2$  was required.<sup>271</sup> Last but not least, the reaction proceeds smoothly with *ortho*-substituted anilines with electron-withdrawing groups such as alkoxycarbonyl, cyano, acetyl and halogens.<sup>271</sup>

## 2.1.4 Hydrolysis and decarboxylation

The last step in the synthetic strategy is a ring opening (hydrolysis) and decarboxylation of the  $\alpha$ -aryl tetramic acid or hydrolysis and decarboxylation of the  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto ester, respectively. It is assumed that ester hydrolysis and decarboxylation of  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto esters would be rather straightforward, whereas ring opening of  $\alpha$ -aryl tetramic acids might be more difficult. Ring opening of tetramic acids is usually performed by a two-step procedure with reduction of the ketone functionality as the first step.<sup>171,272</sup> This indicates that the conjugated system present in tetramic acids makes them more stable than simple lactams. Therefore this section is dedicated to methods for ring opening of tetramic acids rather than methods for hydrolysis/decarboxylation of  $\beta$ -keto ester systems.

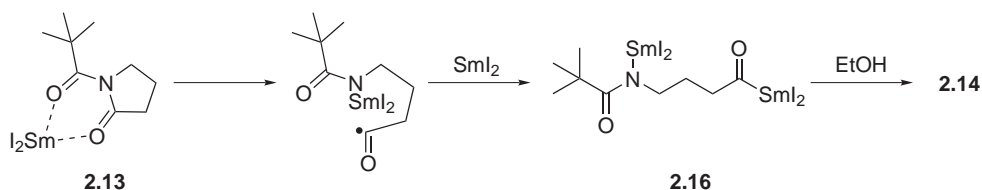
Following the ketone reduction of tetramic acids, which can be accomplished chemically with  $\text{NaBH}_4$  or catalytically with  $\text{H}_2/\text{Pt}_2\text{O}$  (Adam's catalyst),<sup>171,272</sup> they are easily ring





**Scheme 2.1.17:** Treatment of the *N*-acyl lactam **2.13** with  $\text{Sml}_2$  in the presence of a proton donor (EtOH) gave the amidoaldehyde **2.14** in good yield accompanied by minor amounts of the amido ethyl ester **2.15**.<sup>284</sup>

tions; radical cyclizations,<sup>285</sup> Barbier<sup>286–288</sup> and Reformatsky-type reactions,<sup>286,289</sup> conjugated additions,<sup>290</sup> sequential radical processes,<sup>291</sup> radical-anionic sequences<sup>289</sup> and many other radical cyclizations.<sup>292</sup> However, it has never been used for cleavage of tetramic acids. Nevertheless, a  $\text{Sml}_2$ -mediated ring opening of *N*-acyl lactams has been reported<sup>284</sup> and these substrates resemble the *N*-Boc protected tetramic acids a lot (see Scheme 2.1.17).



**Scheme 2.1.18:** Proposed mechanism for the  $\text{Sml}_2$ -mediated ring cleavage of the *N*-acyl lactam **2.13** through a transient acyl samarium species **2.16**, which is trapped by EtOH forming primarily the aldehyde **2.14**.<sup>284</sup>

The *N*-acyl lactam **2.13** was cleaved to the amidoaldehyde **2.14** by treatment with  $\text{Sml}_2$  in the presence of a proton donor (EtOH) in good yield.<sup>284</sup> The amido ethyl ester **2.15** was afforded in minor amounts, probably as a result of nucleophilic attack of the samarium ethoxide on the lactam carbonyl group. These results are similar to those reported for  $\text{Sm}(\text{II})$ -mediated reactions of acid chlorides.<sup>293</sup> They support the hypothesis of the formation of an acyl radical, that is reduced into a transient acyl samarium species **2.16**, which is trapped by an electrophile (EtOH)<sup>284</sup> (see Figure 2.1.18). *N*-Methylsuccinimide and *N*-methylglutarimide were both unreactive under the same experimental conditions, presumably because the ring cleavage of *N*-acyl lactams occurs in a  $\text{Sml}_2$ -acyl lactam complex with samarium chelated by the two carbonyl groups.<sup>284</sup>

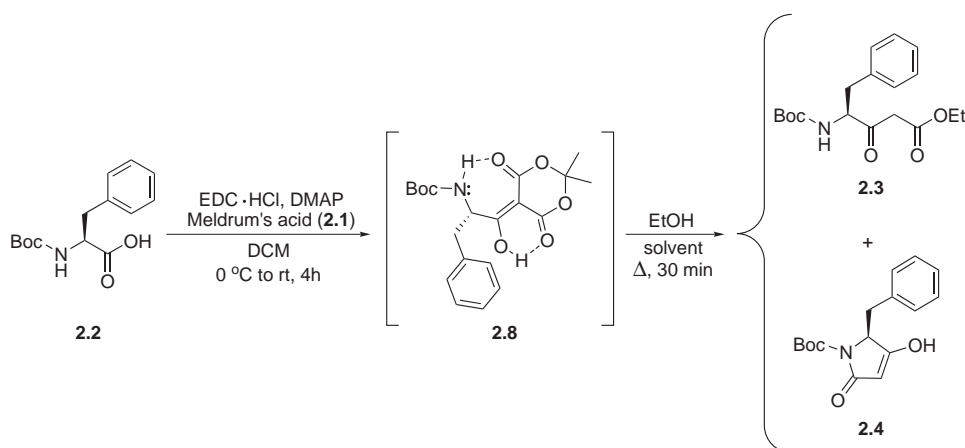
## 2.2 Results and discussion

### 2.2.1 Synthesis of $\gamma$ -amino- $\beta$ -keto esters

#### 2.2.1.1 Optimization of the C-acylation of Meldrum's acid

To optimize the synthesis of the  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** reported by Franck and co-workers,<sup>174</sup> different conditions for work-up of the intermediate **2.8** were tested using pure EtOH or toluene/EtOH (see Table 2.2.1). Boc-Phe-OH (**2.2**) was used as a simple model compound for optimization purposes.

**Table 2.2.1:** Screening of reaction conditions for intermediate **2.8** work-up.



| entry | solvent | EtOH (equiv) | temp. (°C) | <b>2.3</b> : <b>2.4</b> (%) <sup>a</sup> |
|-------|---------|--------------|------------|--|
| 1     | —       | $\infty$     | 80         | 5 : 95                                   |
| 2     | toluene | 2.0          | 110        | 13 : 87                                  |
| 3     | toluene | 2.0          | 80         | 30 : 70                                  |
| 4     | toluene | 4.0          | 80         | 30 : 70                                  |

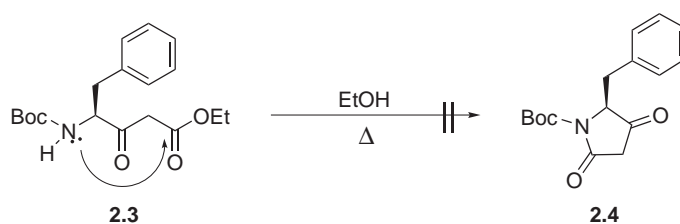
<sup>a</sup>: Ratio between  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** and Boc-*py*Phe-OH (**2.4**) determined by <sup>1</sup>H NMR of the crude product.

Initially, work-up of **2.8** was performed in pure EtOH at reflux (entry 1). Unfortunately, these reaction conditions led to an almost exclusive formation of the Boc-*py*Phe-OH (**2.4**)<sup>a</sup> tetramic acid and only traces of the desired  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** was observed on LC-MS and <sup>1</sup>H NMR.

<sup>a</sup>The prefix '*py*' is used to indicate that the amino acid is converted to a tetramic acid.<sup>211,212,219</sup>

Inspired by Franck and co-workers,<sup>174</sup> who obtained 30% yield of **2.3** by using EtOH (2 equiv) in refluxing benzene, a series of experiments were conducted in toluene (see Table 2.2.1, entries 2–4). Using 2 equiv of EtOH in refluxing toluene<sup>a</sup> gave 13% of **2.3** (see entry 2), but lowering the temperature to 80 °C, which corresponds to the boiling point of benzene, increased the yield to 30% (entry 3). Increasing the equiv of EtOH to 4.0 did not change the yield of **2.3** (see entry 4). Apparently, an intermolecular attack from the amino group is less favored in apolar solvents like benzene and toluene, whereas a polar solvent completely favors an intermolecular attack.

Running reactions at neutral pH might be problematic if more than one product can be formed. To test this, 5% TsOH or 20% pyridine, respectively, was added to toluene experiments. Pyridine did not have any influence on the outcome, whereas TsOH completely decomposed the intermediate **2.8** and neither the  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** nor the tetramic acid **2.4** was formed. Addition of NaOEt (1.10 equiv) was not beneficial either.



**Scheme 2.2.1:** The  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** could not be converted into the corresponding tetramic acid **2.4** by treatment with refluxing EtOH.

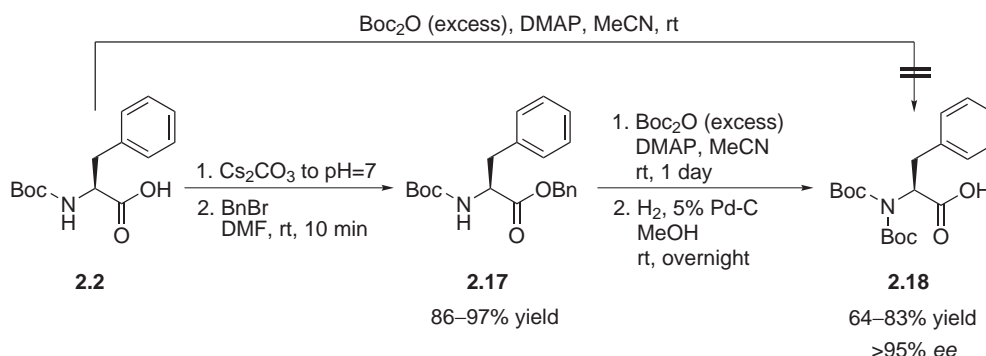
So far, only yields comparable with those obtained by Franck and co-workers<sup>174</sup> were achieved. One plausible explanation for the low yield of the  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** could be the conversion of **2.3** into the tetramic acid **2.4**. Intermolecular attack from the amino group to the carbonyl group would serve as a direct way for formation of **2.4** upon elimination of EtOH. That would represent another path for tetramic acid formation. However, experiments treating the  $\gamma$ -amino- $\beta$ -keto ethyl ester **2.3** with refluxing EtOH did not convert the compound into the corresponding tetramic acid **2.4**. This suggests that whenever **2.3** is formed, it is not converted into the undesired tetramic acid, and that formation of **2.4** happens exclusively through different reaction paths from the common intermediate.

<sup>a</sup>Benzene was substituted with toluene for safety reasons.



### 2.2.1.2 Syntheses of Boc<sub>2</sub>-Phe-OH

To avoid formation of a tetramic acid by cyclization of the intermediate **2.8**, a *N,N*-diprotection approach was investigated. Franck and co-workers<sup>174</sup> demonstrated that *N*-phthaloylglycine exclusively formed the corresponding  $\gamma$ -amino- $\beta$ -keto ester. Although some *N*-phthaloyl protected amino acids are commercially available, functionalized amino acids and in particular the arginine derivative is not available. Furthermore, deprotection is usually performed using hydrazine,<sup>294,295</sup> which is highly toxic and dangerously unstable. Some simple amino acids with an additional *N*-methyl protection are commercially available,<sup>251</sup> but once again functionalized amino acids are absent *N*-demethylation also requires harsh and non-general removal conditions,<sup>296</sup> such as LiClO<sub>4</sub>/O<sub>2</sub>/9,10-dicyanoanthracene/*h* $\nu$ ,<sup>297</sup> *m*-CPBA/FeCl<sub>2</sub>,<sup>298</sup> or PhSeH/160 °C<sup>299</sup> and most of the methods are developed for deprotection of tertiary amines. In addition, *N*-methylamino acids are also enhancedly prone to racemization under basic conditions, probably because of the absence of the *N* $^{\alpha}$ -H, whose ionization would suppress ionization of the neighboring C $^{\alpha}$ -H bond.<sup>300</sup> Imine derivatives is another amine *N,N*-diprotection, which is fairly easy to introduce, but due to their instability they have not been extensively used in synthesis.<sup>301</sup> The most widely used imines are, however, the benzylidene<sup>302</sup> and diphenylmethylene<sup>303,304</sup> imine derivatives, typically introduced by transimination.



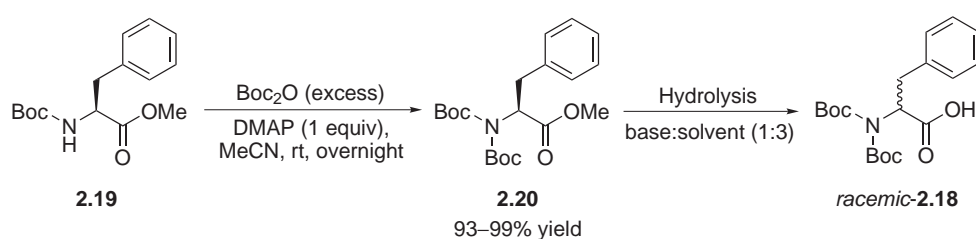
**Scheme 2.2.2:** Synthesis of *N,N*-diprotected Boc<sub>2</sub>-Phe-OH (**2.18**) via the benzyl ester **2.17** in high overall yield and >95% ee determined by <sup>1</sup>H NMR upon derivatization with (*R*)-1-phenyl ethylamine.<sup>305,306</sup> A one-pot approach directly from **2.2** did not work.

Boc-protection is, on the other hand, known as a very convenient protecting group for amines, and therefore a Boc<sub>2</sub>-protection was considered. Gunnarsson and Ragnarsson<sup>306</sup> reported in 1990 a *N,N*-diprotection of amino acids through a temporary benzyl ester protection. Introduction of a second Boc-group has previously been reported to avoid cyclization.<sup>307</sup> Using this approach, Boc-Phe-OH (**2.2**) was protected as the benzyl **2.17**

in yields up to 97% yield (see Figure 2.2.2). Then, the amine was further protected with an additional Boc-group by reaction with excess  $\text{Boc}_2\text{O}$  followed by catalytic hydrogenolysis affording  $\text{Boc}_2\text{-Phe-OH}$  (**2.18**) in high overall yield. A one-pot approach directly from **2.2** using excess of  $\text{Boc}_2\text{O}$  did not form the desired diprotected product **2.18**.

To reduce the number of steps toward to synthesis of  $\text{Boc}_2\text{-Phe-OH}$  (**2.18**), which was selected as the model compound for the proceeding reaction development, another strategy was tested. *N*-Boc  $\alpha$ -amino benzyl esters (such as **2.17**) are not commercially available, but the methyl esters are. By using a preprotected compound, the sequence could be reduced by one step.  $\text{Boc-Phe-OMe}$  (**2.19**) was initially Boc-protected with an excess amount of  $\text{Boc}_2\text{O}$  affording the  $\text{Boc}_2\text{-Phe-OMe}$  (**2.20**) in excellent yield. Then, a series of hydrolysis experiments were set up to find optimized conditions for preparation of  $\text{Boc}_2\text{-Phe-OH}$  (**2.18**) (see Table 2.2.2).

**Table 2.2.2:** Testing of hydrolysis conditions for *O*-deprotection of **2.20**.



| entry | base <sup>a</sup> | solvent | temp. (°C)     | time (h) | yield (%) <sup>b</sup> | ee (%) <sup>c</sup> |
|-------|-------------------|---------|----------------|----------|------------------------|---------------------|
| 1     | LiOH              | THF     | rt to $\Delta$ | 16       | 70 <sup>d</sup>        | – <sup>e</sup>      |
| 2     | LiOH              | MeOH    | $\Delta$       | 0.5      | 77 <sup>d</sup>        | – <sup>e</sup>      |
| 3     | LiOH              | MeOH    | rt             | 16       | 88                     | 0                   |
| 4     | NaOH              | MeOH    | rt             | 0.5      | 85                     | 0                   |
| 5     | NaOH              | MeOH    | 5              | 7.5      | 91                     | 0                   |

<sup>a</sup>: Saturated aqueous solution. <sup>b</sup>: Isolated yield. <sup>c</sup>: Enantiomeric excess determined by coupling of product with (*R*)-1-phenyl ethylamine and subsequently analyzed by <sup>1</sup>H NMR. <sup>d</sup>: Impure product. <sup>e</sup>: ee not determined due to impure products.

LiOH in THF was tested (entry 1), but the hydrolysis was very sluggish and had to be heated to reflux to secure conversion of the starting material. Unfortunately, the product **2.18** was quite impure. The same result was obtained with LiOH in MeOH at reflux, which gave full conversion after only 30 minutes (entry 2). Lowering the temperature to room temperature gave 88% yield of pure  $\text{Boc}_2\text{-Phe-OH}$  (**2.18**) after 16 hours, but unfortunately, complete racemization had occurred (entry 3). NaOH was tested as an alternative to avoid racemization. This base hydrolyzed the methyl ester **2.20** much faster

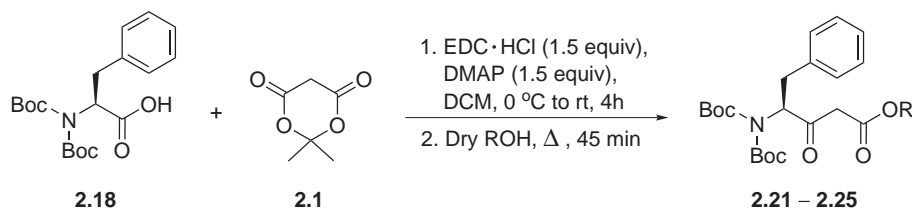
than LiOH, and the reaction was complete after just 30 minutes at room temperature. Unfortunately, complete racemization was observed (entry 4). Further temperature reduction to 5 °C, did still not give any noteworthy enantiomeric excess (entry 5).

Deprotection of Boc-Phe-OMe (**2.19**) was tested using NaOH in MeOH at room temperature, which did not lead to any racemization. This result indicates clearly that the *N,N*-diprotected amino acids are much more prone to racemization, apparently because of an increased acidity of the  $\alpha$ -proton. Furthermore, it was discovered, that the two Boc-groups did not have the same lability. The second Boc-group was much more labile than the first one, which might be due to electronic and steric effects.

### 2.2.1.3 Utilization of Boc<sub>2</sub>-Phe-OH in $\beta$ -keto ester synthesis

Using the optimized reaction conditions developed for the tetramic acid synthesis,<sup>211,212,219</sup> Boc<sub>2</sub>-Phe-OH (**2.18**) was applied for the synthesis of a range of  $\gamma$ -amino- $\beta$ -keto esters **2.21–2.25** (see Table 2.2.3).

**Table 2.2.3:** Synthesis of  $\gamma$ -amino- $\beta$ -keto esters.



| entry | R           | product     | yield (%) <sup>a</sup> |
|-------|-------------|-------------|------------------------|
| 1     | Me          | <b>2.21</b> | 94                     |
| 2     | Et          | <b>2.22</b> | 95                     |
| 3     | <i>i</i> Pr | <b>2.23</b> | 98                     |
| 4     | <i>t</i> Bu | <b>2.24</b> | 97                     |
| 5     | Bn          | <b>2.25</b> | (>95) <sup>b</sup>     |

<sup>a</sup>: Isolated yield. Purity determined by HPLC to be greater than 95%. <sup>b</sup>: Conversion determined by TLC, but otherwise not characterized.

Initially it was observed that **2.18** reacted slightly slower compared to the mono-protected amino acid, Boc-Phe-OH (**2.2**). The reaction rate was improved by increasing the equivs of EDC and **2.1** from 1.13 to 1.50. Furthermore, it was found that the intermediate formed from **2.18** was less stable compared to **2.8**, therefore immediate work-up with cold solvents is recommended. Last but not least, small amounts of the methyl ketone derivative of **2.18** was observed in the product (5–12%), which was probably due to hydrolysis of the  $\beta$ -keto ester followed by decarboxylation. Formation of this methyl ketone was suppressed by using dried alcohols in the work-up step. Increased reaction times up to 45 minutes secured full conversion.

With these optimized conditions five different *N,N*-diprotected  $\gamma$ -amino- $\beta$ -keto esters **2.21–2.24** were synthesized in good yield (94–98%, entries 1–4). The additional Boc-group was stable under the chosen reaction conditions. No difference in product yields was observed among the different alcohols tested, despite that fact that MeOH (entry 1) is a better nucleophile than *t*BuOH (entry 4). The *N,N*-diprotected  $\gamma$ -amino- $\beta$ -keto benzyl ester **2.25** (entry 5) was also easily formed, but isolation of the product was complicated by the high boiling point of BnOH. Based on the previous results, it seems evident that the  $\beta$ -keto benzyl ester also could be formed by treating the intermediate **2.8** with toluene and BnOH (2 equiv) at reflux. However, this method was not tested.

#### 2.2.1.4 Application of strategy to functionalized amino acids

So far, the method has only been applied to the simple Boc<sub>2</sub>-Phe-OH (**2.18**). Therefore, the strategy was tested with more functionalized amino acids. Initially a range of carboxylic protected amino acids were synthesized (see Table 2.2.4).

The benzyl ester was once again utilized (entries 1–3), but for the compounds with Cbz-protection on the side-chain, an allyl ester was chosen (entries 4–6). This protection was introduced by using allylbromide.<sup>306</sup> The allyl carboxylic protection can be removed orthogonally to the Cbz-protection by treatment with Rh(PPh<sub>3</sub>)<sub>3</sub>.<sup>308</sup>

Introduction of the benzyl or allyl esters proceeded nicely for a wide range of functionalized amino acids in good yields (see Table 2.2.4). Basically, the two protection groups were introduced equally easy, however, the allylbromide reacted somewhat slower than benzylbromide.

**Table 2.2.4:** *Carboxylic protection of functionalized amino acids.*

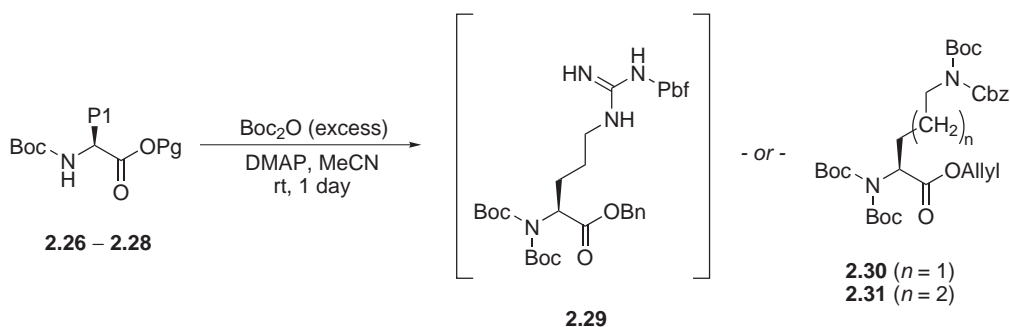


| <i>entry</i> | <i>side-chain (P1)<sup>a</sup></i> | <i>route<sup>b</sup></i> | protection (Pg) | <i>product</i> | <i>yield (%)<sup>c</sup></i> |
|--------------|------------------------------------|--------------------------|-----------------|----------------|------------------------------|
| 1            | Arg(Pbf)                           | A                        | Bn              | <b>2.26</b>    | 80–88                        |
| 2            | Orn(Cbz)                           | B                        | Allyl           | <b>2.27</b>    | 92                           |
| 3            | Lys(Cbz)                           | B                        | Allyl           | <b>2.28</b>    | 95                           |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain (P1). <sup>b</sup>: Route A corresponds to benzyl protection with BnBr, whereas route B corresponds to allyl protection with allylbromide. <sup>c</sup>: Isolated yield.

With the carboxylic protected amino acids in hand, the introduction of the additional  $N^\alpha$ -protection was tested for a variety of different substrates (see Table 2.2.5). As with the Boc-Phe-OBn (**2.17**), the Boc-group was projected to be introduced with an excess of  $\text{Boc}_2\text{O}$ .

**Table 2.2.5:** *Synthesis of fully protected functionalized amino acids.*



| <i>entry</i> | <i>substrate (P1, Pg)</i>           | <i>product</i> | <i>yield (%)<sup>a</sup></i> |
|--------------|-------------------------------------|----------------|------------------------------|
| 1            | Boc-Arg(Pbf)-OBn ( <b>2.26</b> )    | <b>2.29</b>    | — <sup>b</sup>               |
| 2            | Boc-Orn(Cbz)-OAllyl ( <b>2.27</b> ) | <b>2.30</b>    | 72                           |
| 3            | Boc-Lys(Cbz)-OAllyl ( <b>2.28</b> ) | <b>2.31</b>    | 73                           |

<sup>a</sup>: Isolated yields. <sup>b</sup>: Product was not isolated due to formation of many undefined products and severe contamination of Boc<sub>2</sub>O.

Unfortunately, introduction of an additional  $N^\alpha$ -protection was not straightforward when several nucleophilic nitrogen atoms were present in the molecule. Apparently no difference in reactivity was observed among the  $N^\alpha$  and the side-chain nitrogen atom, e.g. in the guanine group ( $N^\omega$ ) or the lysine group ( $N^\epsilon$ ), although these functionalities were appropriately protected with Cbz or Pbf (see Table 2.2.5). This resulted in mixtures of products with different degrees of Boc-protection, ranging from introduction of only one additional Boc-group, with unknown regioselectivity, to many additional Boc-groups. Because of these problems with regioselectivity, the many compounds formed upon treatment of Boc-Arg(Pbf)-OBn (**2.26**) with  $\text{Boc}_2\text{O}$  were not further isolated (entry 1). Such a procedure would never be desirable and does indeed not represent a convenient and rapid procedure for  $N,N$ -diprotected amino acids. Introduction of the additional Boc-group was however easier with Boc-Orn(Cbz)-OAllyl (**2.27**) and Boc-Lys(Cbz)-OAllyl (**2.28**), which simply resulted in the fully protected amino acids **2.30** and **2.31**, respectively (entries 2 and 3). For both of these compounds it was possible to force the reaction to completion and one defined product could be obtained.

Further optimization of the chemistry regarding the  $\gamma$ -amino- $\beta$ -keto esters was discontinued at this point. This key intermediate seemed to be more difficult to synthesize via Meldrum's acid (**2.1**) than expected, and synthesis of  $N,N$ -diprotected amino acids was uphill when functionalized side-chains were present. The traditional syntheses of  $\gamma$ -amino- $\beta$ -keto esters requiring the use of organometallic reagents in excess, would neither be an alternative because of the resulting low functional group tolerance.

#### 2.2.1.5 Summary

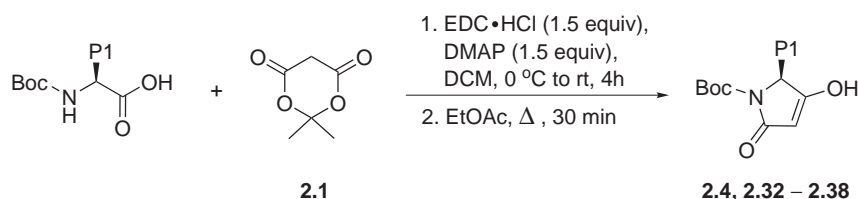
A mild synthesis of amino acid-derived  $\gamma$ -amino- $\beta$ -keto esters has been developed. In this way, a range of phenylalanine-derived  $\beta$ -keto esters **2.21–2.24** were synthesized in excellent yield (94–98%). The method does not require strong bases which is usually employed for synthesis of such compounds. Instead, the synthesis employs the  $N,N$ -diprotected amino acid  $\text{Boc}_2\text{-Phe-OH}$  (**2.18**) and commercially available Meldrum's acid (**2.1**).  $\text{Boc}_2\text{-Phe-OH}$  (**2.18**) was synthesized in three steps in good overall yield (up to 80%) with no racemization. Unfortunately, this method seemed only really applicable for non-functionalized amino acids due to poor regioselectivity of the additional Boc-group introduction. Synthesis of  $\text{Boc}_2\text{-Arg(Pbf)-OH}$  (**2.29**), which is crucial for applicability of the chemistry for preparation of the desired arginyl benzyl ketone building block, was found to be unsuitable. In spite of several attempts, optimization of the synthesis of  $\gamma$ -amino- $\beta$ -keto esters from mono-protected amino acids failed, and only up to 30% of the desired product was isolated. Apparently formation of the tetramic acid is highly favored and very difficult, if not impossible, to avoid.

## 2.2.2 Synthesis of tetramic acids

Based on how easily amino acid-derived tetramic acids are synthesized from *N*-Boc-protected amino acids and Meldrum's acid (**2.1**), the strategy using this key intermediate seemed to be more likely to succeed.

A variety of different tetramic acids were therefore synthesized in excellent yield according to literature procedures.<sup>171–173,211,212,219</sup> EDC was selected as coupling reagent due to the easy work-up procedure using diluted acid for removal of the corresponding urea by-product.

**Table 2.2.6:** Synthesis of amino acid-derived tetramic acids.



| entry | side-chain (P1) <sup>a</sup> | product     | yield (%) <sup>b</sup> |
|-------|------------------------------|-------------|------------------------|
| 1     | Phe                          | <b>2.4</b>  | 76–98                  |
| 2     | Arg(Pbf)                     | <b>2.32</b> | 77–99                  |
| 3     | Tyr( <sup>t</sup> Bu)        | <b>2.33</b> | quant. <sup>c</sup>    |
| 4     | Lys(Cbz)                     | <b>2.34</b> | 78–99                  |
| 5     | Thr( <sup>t</sup> Bu)        | <b>2.35</b> | quant. <sup>c</sup>    |
| 6     | Asp( <sup>t</sup> Bu)        | <b>2.36</b> | 94                     |
| 7     | Gly                          | <b>2.37</b> | 99                     |
| 8     | Orn(Cbz)                     | <b>2.38</b> | 94%                    |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain (P1). <sup>b</sup>: Isolated yield. <sup>c</sup>: Yield was determined to be >100% because of solvent contaminations which could not be removed in high vacuum.

A variety of different tetramic acids was synthesized in excellent yield and purity with no need of flash chromatography. Compared to the previously described facile synthesis of  $\gamma$ -amino- $\beta$ -keto esters, tetramic acids are formed very easily with no significant competing by-product formation. Furthermore, the chemistry was compatible with a variety of different functionalities and protecting groups. Boc-*py*Arg(Pbf)-OH (**2.32**) which is the most interesting tetramic acid for the arginyl benzyl ketone building block synthesis, did also form smoothly (see Table 2.2.6, entry 2).

### 2.2.3 Palladium-catalyzed $\alpha$ -arylation of tetramic acids

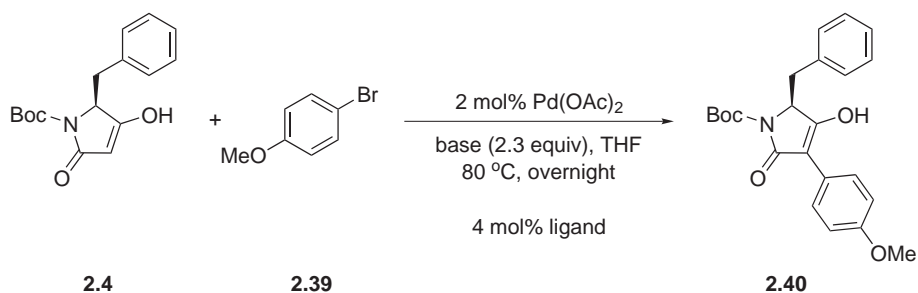
With the access to a variety of different amino acid-derived tetramic acids, the subsequent  $\alpha$ -arylation was then investigated. Palladium-catalyzed cross-couplings usually require extensive screening and optimization and therefore it was decided to base the experiments on the general trends in  $\alpha$ -arylation of carbonyl compounds, as described in section 2.1.3. This transformation is not only one of the key steps in the application of tetramic acids for the synthesis of the benzyl ketone building block, but also a new possible way for preparation of  $\alpha$ -aryl tetramic acids. These compounds are themselves interesting due to their potential biological activity.

#### 2.2.3.1 Initial screening of bases and ligands

Initially, arylation of Boc-*py*Phe-OH (**2.4**) with 4-bromoanisole (**2.39**) in the presence of 2 mol% Pd(OAc)<sub>2</sub> was tested for the synthesis of the  $\alpha$ -aryl tetramic acid **2.40** (see Table 2.2.7).

Four different weak, inorganic bases were tested; Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>, respectively, in 2.3 equivs inspired by results published by Buchwald and co-workers.<sup>230</sup> Screening of a variety of weak bases was prioritized because they are much more compatible with functional groups, and because tetramic acids are only slightly acidic ( $pK_a \approx 6.4$ ).<sup>199,200</sup>

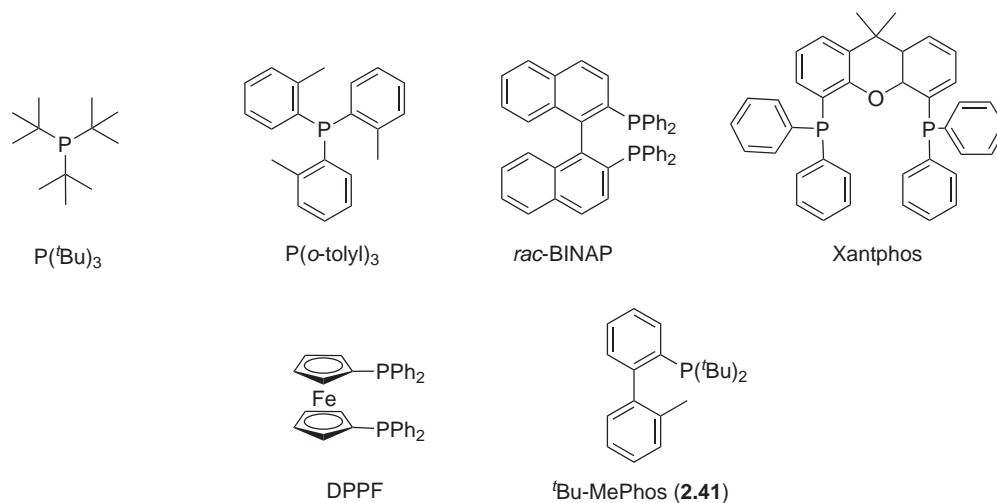


**Table 2.2.7:** Results from initial screening of bases and ligands.

| entry | ligand                                 | base                            | conv. (%) <sup>a</sup> |
|-------|--|---------------------------------|------------------------|
| 1     | P( <sup>t</sup> Bu) <sub>3</sub>       | all four <sup>b</sup>           | <5                     |
| 2     | P( <i>o</i> -tolyl) <sub>3</sub>       | all four <sup>b</sup>           | <5                     |
| 3     | <i>rac</i> -BINAP                      | all four <sup>b</sup>           | <5                     |
| 4     | Xantphos                               | all four <sup>b</sup>           | <5                     |
| 5     | DPPF                                   | all four <sup>b</sup>           | <5                     |
| 6     | <sup>t</sup> Bu-MePhos ( <b>2.41</b> ) | Cs <sub>2</sub> CO <sub>3</sub> | 15                     |
| 7     | <sup>t</sup> Bu-MePhos ( <b>2.41</b> ) | K <sub>3</sub> PO <sub>4</sub>  | 36                     |
| 8     | <sup>t</sup> Bu-MePhos ( <b>2.41</b> ) | Na <sub>2</sub> CO <sub>3</sub> | — <sup>c</sup>         |
| 9     | <sup>t</sup> Bu-MePhos ( <b>2.41</b> ) | K <sub>2</sub> CO <sub>3</sub>  | 36                     |

<sup>a</sup>: Determined by <sup>1</sup>H NMR. <sup>b</sup>: Ligand tested with Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>, respectively. <sup>c</sup>: No significant product formed based on <sup>1</sup>H NMR and LC-MS.

The phosphine ligands P(<sup>t</sup>Bu)<sub>3</sub>, P(*o*-tolyl)<sub>3</sub>, *rac*-BINAP, Xantphos and DPPF (see Figure 2.2.1) were screened with the four bases in 20 initial experiments (see Table 2.2.7, entries 1–5). Unfortunately, none of these conditions gave rise to any significant product formation (conv.: <5%) and only starting materials were isolated upon acidic work-up. However, screening experiments with 4 mol% <sup>t</sup>Bu-MePhos **2.41**, a biaryl phosphine ligand, gave promising results (entries 6–9). Not surprisingly, a major difference among the bases was observed, K<sub>3</sub>PO<sub>4</sub> and K<sub>2</sub>CO<sub>3</sub> gave comparable results, 36% conversion (entries 7 and 9), whereas Na<sub>2</sub>CO<sub>3</sub> did not give any product formation at all (entry 8). Cs<sub>2</sub>CO<sub>3</sub> gave a modest result with a conversion of 15% (entry 6).



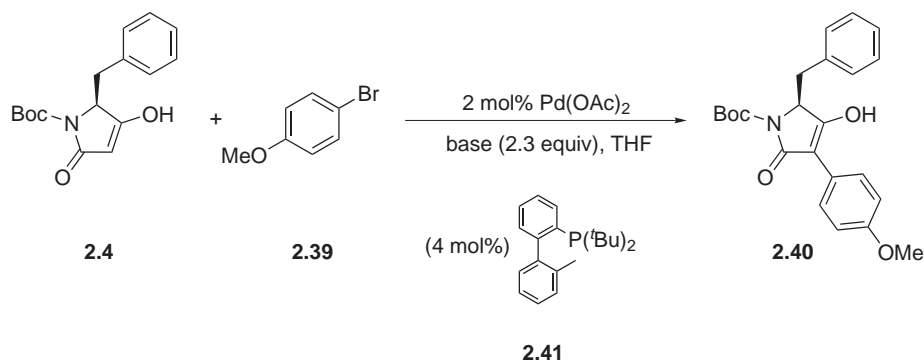
**Figure 2.2.1:** Ligands used in the initial screening experiments.

### 2.2.3.2 Variation of temperature and equivalents

To further increase the conversion with 4 mol% *t*-Bu-MePhos **2.41** and 2.3 equiv  $K_3PO_4$ , a series of experiments at elevated temperature (100 °C) and a series with prolonged reaction time (3 days) were conducted (see Table 2.2.8). No significant change in conversions was observed after 3 days (entries 1–4) at 80 °C. On the other hand, the conversion was increased at 100 °C overnight, especially with  $K_3PO_4$  which almost gave a two-fold increase in conversion to 71% (entry 6). In both experimental series, the use of  $Na_2CO_3$  still did not give any significant product formation (entries 3 and 7). Furthermore, experiments at room temperature were conducted but no product formation was observed.

Inefficient activation of the catalyst may cause the low to moderate conversions obtained so far. Therefore the screening reaction was tested at 80 °C with all four bases, respectively, with 2 mol%  $Pd_2(dba)_3$  as a direct source of Pd(0). However, there was no improvement in conversion, and it is therefore assumed that the problem with low conversion is not due to the nature of the palladium catalyst.

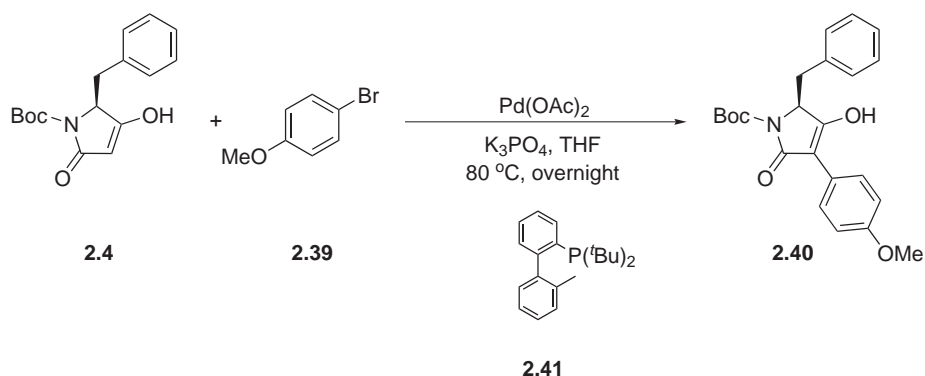
Having a set of reaction conditions giving a moderate conversion and a catalyst that presumably is sufficiently activated, a set of different equiv with regard to 4-bromoanisole (**2.39**),  $Pd(OAc)_2$ , ligand **2.41** and  $K_3PO_4$  was screened (see Table 2.2.9). Increasing the equiv of **2.39** from 1.0 to 2.0 only increased the conversion slightly (entry 1), whereas increasing the equiv of  $K_3PO_4$  to 5.0 gave a significant reduction in conversion (entry 2). Furthermore, the effect of catalyst and ligand loading was examined. Increasing the

**Table 2.2.8:** Increased temperature and prolonged reaction time.

| entry | base                            | temp (°C) | time (h) | conv. (%) <sup>a</sup> |
|-------|---------------------------------|-----------|----------|------------------------|
| 1     | Cs <sub>2</sub> CO <sub>3</sub> | 80        | 72       | 21                     |
| 2     | K <sub>3</sub> PO <sub>4</sub>  | 80        | 72       | 33                     |
| 3     | Na <sub>2</sub> CO <sub>3</sub> | 80        | 72       | <5                     |
| 4     | K <sub>2</sub> CO <sub>3</sub>  | 80        | 72       | 32                     |
| 5     | Cs <sub>2</sub> CO <sub>3</sub> | 100       | 16       | 25                     |
| 6     | K <sub>3</sub> PO <sub>4</sub>  | 100       | 16       | 71                     |
| 7     | Na <sub>2</sub> CO <sub>3</sub> | 100       | 16       | <5                     |
| 8     | K <sub>2</sub> CO <sub>3</sub>  | 100       | 16       | 52                     |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

loading of catalyst and ligand to 4 and 8 mol%, respectively, gave full conversion (entry 3). The same was true with increased ligand loading only (entry 4). It was found that an excess of ligand was essential since 4 mol% Pd(OAc)<sub>2</sub> and 4 mol% *t*Bu-MePhos **2.41** only gave a conversion of 79% (entry 5).

**Table 2.2.9:** Variation of equivalents.

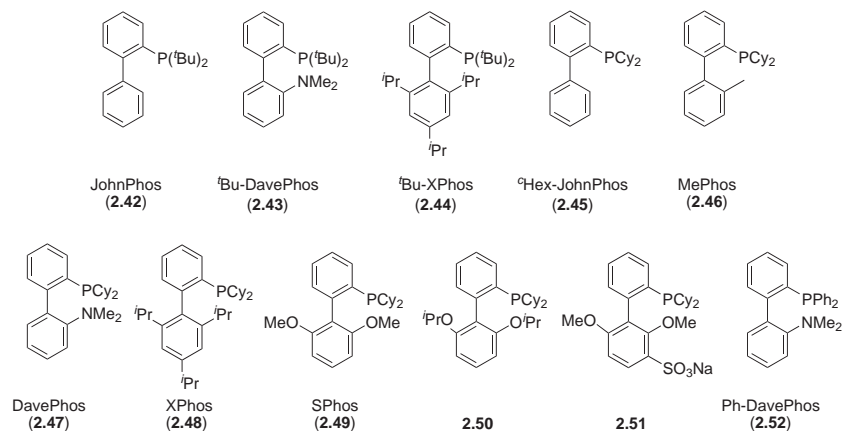
| entry | Ar-Br <b>2.39</b><br>(equiv) | $\text{Pd}(\text{OAc})_2$<br>(mol%) | ligand <b>2.41</b><br>(mol%) | $\text{K}_3\text{PO}_4$<br>(equiv) | conv.<br>(%) <sup>a</sup> |
|-------|------------------------------|-------------------------------------|------------------------------|------------------------------------|---------------------------|
| 1     | 2.0                          | 2                                   | 4                            | 2.3                                | 43                        |
| 2     | 1.0                          | 2                                   | 4                            | 5.0                                | 15                        |
| 3     | 1.0                          | 4                                   | 8                            | 2.3                                | >95                       |
| 4     | 1.0                          | 2                                   | 8                            | 2.3                                | >95                       |
| 5     | 1.0                          | 4                                   | 4                            | 2.3                                | 79                        |

<sup>a</sup> Determined by  $^1\text{H}$  NMR.

### 2.2.3.3 Screening of biaryl phosphine ligands

Based on these results, it seemed likely that screening other biaryl phosphine ligands might give full conversion without increasing the ligand loading. Three classes of commercially available biaryl phosphine ligands were therefore screened; a series of di-*tert*-butyl biaryl phosphines **2.42**–**2.44**, a series of dicyclohexyl biaryl phosphines **2.45**–**2.51** and one diphenyl biaryl phosphine, the Ph-DavePhos (**2.52**) (see Figure 2.2.2). Buchwald and co-workers<sup>309</sup> reported in 2000 an improved and efficient one-pot synthesis of functionalized dicyclohexyl- and di-*tert*-butylphosphinobiphenyl ligands,<sup>309</sup> however the presented ligands here are all commercially available.<sup>310</sup>

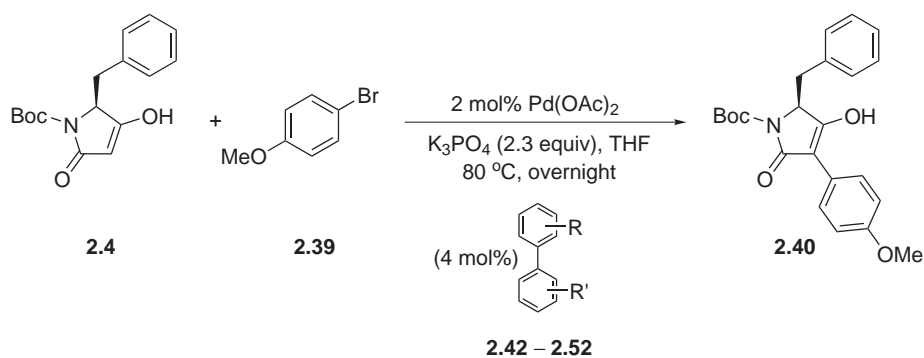
Only *t*Bu-XPhos (**2.44**) gave full conversion with 4 mol% ligand loading (see Table 2.2.10, entry 3). The two other di-*tert*-butyl biaryl phosphines, JohnPhos (**2.42**) and *t*Bu-DavePhos (**2.43**) gave only poor conversion (entries 1 and 2). All dicyclohexyl biaryl phosphine ligands **2.45**–**2.51** (with interesting names such as MePhos, DavePhos, XPhos and SPhos) and the Ph-DavePhos ligand **2.52** gave only traces of product. Apparently, this reaction requires a sterically hindered ligand, and the di-*tert*-butyl substituents



**Figure 2.2.2:** Biaryl phosphine ligands; di-*tert*-butyl (2.42–2.44) and dicyclohexyl (2.45–2.51) ligands and a diphenyl ligand (2.52).

are essential for reactivity. This was demonstrated by the absence of reactivity with the analogous dicyclohexyl biaryl phosphine ligand, XPhos (2.48).

**Table 2.2.10:** Screening of different biaryl phosphine ligands.



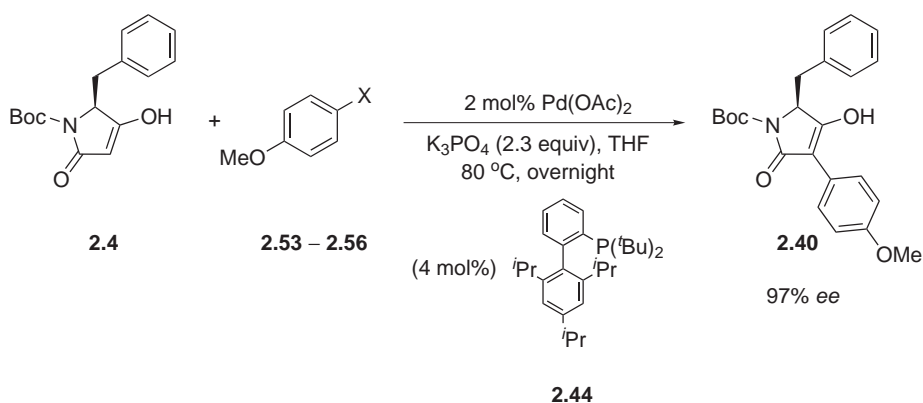
| entry | ligand                 | conv. (%) <sup>a</sup> |
|-------|------------------------|------------------------|
| 1     | 2.42                   | 5                      |
| 2     | 2.43                   | 21                     |
| 3     | 2.44                   | >95                    |
| 4     | 2.45–2.52 <sup>b</sup> | <5                     |

<sup>a</sup> Determined by <sup>1</sup>H NMR. <sup>b</sup>: Tested individually in 8 different experiments.

### 2.2.3.4 Substrate scope and limitations

Having arrived at these optimized reaction conditions it would be desirable to examine the scope and limitations of the reaction. This was initially done by testing other different aryl coupling partners, namely aryl chlorides, iodides, tosylates and triflates. For comparison reasons it was chosen to screen 4-methoxy derivatives only (see Table 2.2.11). It was delightful to discover that 4-chloroanisole (**2.53**) reacted identically compared to 4-bromoanisole (**2.39**) (entry 1), as did the aryl triflate **2.56** (entry 4). Use of aryl triflates expands the scope of the reaction further because it allows conversion of phenols into functional coupling partners very easily. Aryl iodide **2.54** and tosylate **2.55** gave only minor amounts or traces of product **2.40**, respectively (entries 2 and 3). This observation is important because aryl chlorides are generally much cheaper than the corresponding iodides and a wider range of commercially available compounds exists. Moreover, this chemistry shows a reverse reactivity as aryl chlorides are usually too unreactive for palladium-catalyzed coupling reactions.

**Table 2.2.11:** Testing of different coupling partners.

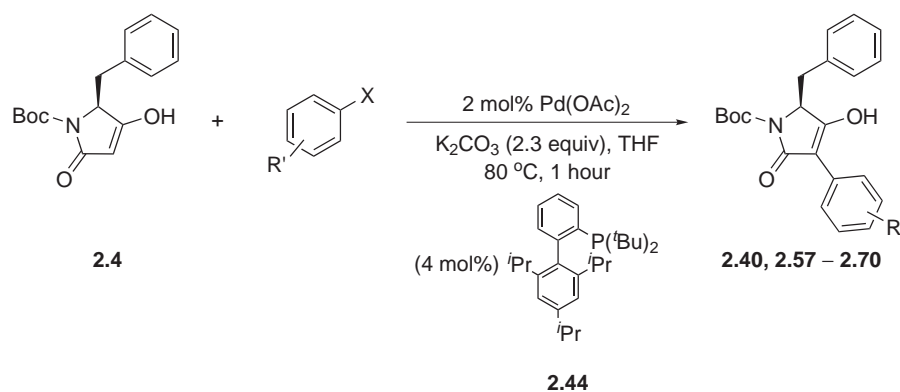


| entry | X                   | conv. (%) <sup>a</sup> |
|-------|---------------------|------------------------|
| 1     | Cl ( <b>2.53</b> )  | >95                    |
| 2     | I ( <b>2.54</b> )   | <5                     |
| 3     | OTs ( <b>2.55</b> ) | 8                      |
| 4     | OTf ( <b>2.56</b> ) | >95                    |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

Using the optimized reaction conditions a broad range of aryl chlorides were tested with different substituents, electron-donating (EDG) as well as electron-withdrawing groups (EWG), and with different disubstitution patterns (see Table 2.2.12).

**Table 2.2.12:** *Substrate scope with different aryl chlorides.*



| entry | $R'$ ( $X=\text{Cl}$ )                          | time (h) | product                  | yield (%) <sup>a</sup> |
|-------|---|----------|--------------------------|------------------------|
| 1     | H   | 1        | <b>2.57</b>              | 75                     |
| 2     | 4-OMe   | 1        | <b>2.40</b>              | 79                     |
| 3     | 3-OMe   | 1        | <b>2.58</b>              | 78                     |
| 4     | 2-OMe   | 16       | <b>2.59</b>              | – <sup>b</sup>         |
| 5     | 2-OMe ( $X=\text{Br}$ )                         | 1        | <b>2.59</b>              | – <sup>b</sup>         |
| 6     | 4-OH  | 16       | <b>2.60</b>              | 72                     |
| 7     | 4-NH <sub>2</sub>                               | 16       | <b>2.61</b>              | (>95%) <sup>c</sup>    |
| 8     | 4-NHBoc   | 1        | <b>2.62</b>              | 75                     |
| 9     | 4-C <sub>2</sub> H <sub>4</sub> NH <sub>2</sub> | 1        | <b>2.63</b>              | – <sup>b</sup>         |
| 10    | 4-CN  | 1        | <b>2.64</b>              | 74                     |
| 11    | 2-CN  | 16       | <b>2.65</b>              | – <sup>b</sup>         |
| 12    | 4-NO <sub>2</sub>                               | 16       | <b>2.66</b>              | 60                     |
| 13    | 4-COCH <sub>3</sub>                             | 1        | <b>2.67</b>              | 74                     |
| 14    | 4-COOH  | 16       | <b>2.68</b>              | (>95%) <sup>c</sup>    |
| 15    | 4-COOMe   | 1        | <b>2.69</b>              | 75                     |
| 16    | 4-Br  | 1        | <b>2.70</b> <sup>d</sup> | 77                     |

<sup>a</sup> Purified by flash chromatography. <sup>b</sup> No significant product formation determined by <sup>1</sup>H NMR or LC-MS. <sup>c</sup> Not purified by flash chromatography, but conversion determined by crude <sup>1</sup>H NMR. <sup>d</sup> Only the 4-chlorobenzene product was observed determined by LC-MS.

Both *meta*- and *para*-disubstituted aryl chlorides reacted efficiently giving full conversion after just 1 hour for most of the substrates. Electron-donating groups such as ethers,

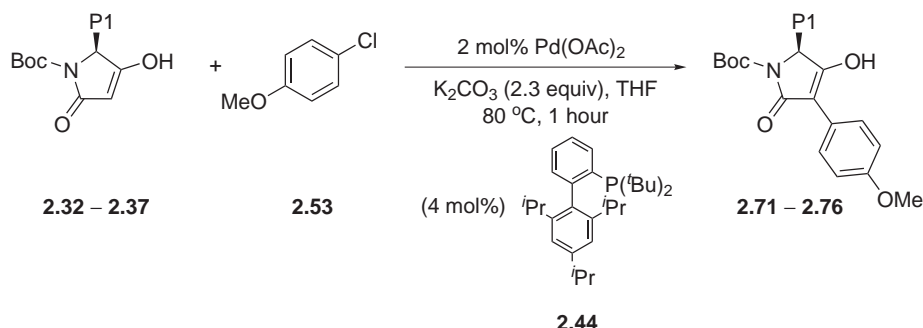
alcohols and amines (entries 3, 6 and 7) worked well and the same was true for a variety of electron-withdrawing groups like nitriles, nitro groups, ketones and esters (entries 10, 12, 13 and 15). Chlorobenzene itself also reacted smoothly giving full conversion after 1 hour (entry 1). Coupling of 4-chlorophenol, 4-chloroaniline, 4-chloronitrobenzene and 4-chlorobenzoic acid did not proceed to completion after 1 hour (entries 6, 7, 12 and 14), but full conversion was achieved overnight (16 hours). Apparently, the unprotected functional groups slowed down the reaction. Protection of the aniline nitrogen as in (*N*-Boc)-4-chloro-aniline gave full conversion after 1 hour (entry 8). In the case of a free aliphatic amine (entry 9), the unprotected nitrogen completely quenched the reaction. To synthesize a halogen-substituted product, the chemistry with 1-bromo-4-chlorobenzene (entry 16) was tested and the 4-chloro product **2.80** was formed exclusively. This is reasonable because bromides react faster than chlorides. Finally, a couple of *ortho*-substituted aryl chlorides were examined, but none of them gave any significant product formation after 16 hours (entries 4 and 11). For *ortho*-methoxy substitution, 2-bromoanisole was tested with the more reactive bromide (entry 5), but no reaction was observed. It is not surprising that *ortho*-substituted aryl halides did not react at all, since there is much more steric hindrance around the halogen on these coupling partners. The enantiomeric purity of **2.40** was analyzed by means of chiral HPLC. Fortunately, only minor racemization had occurred (97% ee), which was expected due to the use of mild base.

Besides of the aryl chlorides, the scope of the reaction was also examined with a series of heterocycles. Three different chloro-pyridines, 2-chloropyrimidine, 5-chloro-1-methyl-1*H*-imidazole, two chloro-thiophenes and 3-bromothiophene were tested. Unfortunately, none of them gave any significant formation of product after 16 hours. It is plausible that the heterocycles simply coordinate to palladium resulting in an unreactive complex.

Finally, the scope of the reaction was expanded by testing other tetramic acids than Boc-*py*Phe-OH (**2.4**). A series of functionalized tetramic acids were chosen; Boc-*py*Arg(Pbf)-OH (**2.32**), Boc-*py*Tyr(*t*Bu)-OH (**2.33**), Boc-*py*Lys(Cbz)-OH (**2.34**), Boc-*py*Thr(*t*Bu)-OH (**2.35**), Boc-*py*Asp(*t*Bu)-OH (**2.36**) and Boc-*py*Gly-OH (**2.37**). These were all subjected to the optimized reaction conditions with 4-chloroanisole (**2.53**) as the coupling partner (see Table 2.2.13). The chosen tetramic acids represent a broad variety of functional side-chains and different protecting groups. Most of them gave similar yields compared to the simpler phenylalanine model compound. Boc-*py*Asp(*t*Bu)-OH (**2.36**) and especially Boc-*py*Gly-OH (**2.37**) gave however much lower yield of the corresponding  $\alpha$ -aryl tetramic acids **2.75** and **2.76**, respectively (entries 5 and 6).

The reaction between Boc-*py*Phe-OH (**2.4**) and 4-chloroanisole (**2.53**) was tested with microwave heating and it was found that full conversion (>95%) was achieved within only 5 minutes at 110 °C.



**Table 2.2.13:** *Substrate scope with different tetramic acids.*

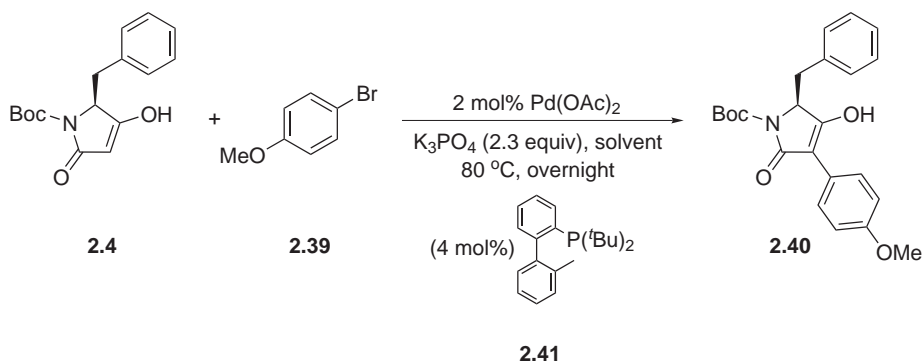
| entry | side-chain (P1) <sup>a</sup>          | time (h) | product     | yield (%) <sup>b</sup> |
|-------|---------------------------------------|----------|-------------|------------------------|
| 1     | Arg(Pbf) ( <b>2.32</b> )              | 16       | <b>2.71</b> | 67                     |
| 2     | Tyr( <sup>t</sup> Bu) ( <b>2.33</b> ) | 1        | <b>2.72</b> | 69                     |
| 3     | Lys(Cbz) ( <b>2.34</b> )              | 1        | <b>2.73</b> | 69                     |
| 4     | Thr( <sup>t</sup> Bu) ( <b>2.35</b> ) | 1        | <b>2.74</b> | 62                     |
| 5     | Asp( <sup>t</sup> Bu) ( <b>2.36</b> ) | 16       | <b>2.75</b> | 45                     |
| 6     | Gly ( <b>2.37</b> )                   | 16       | <b>2.76</b> | 28 <sup>c</sup>        |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain (P1). <sup>b</sup>: Purified by flash chromatography. <sup>c</sup>: Flash chromatography did not successfully purify the product.

### 2.2.3.5 Solvent screening

So far, all reactions were conducted in THF. The initial reaction was revisited and other solvents were tested (see Table 2.2.14). Dioxane, MeCN and DMF gave only traces of product (<5%) no matter which base was used (Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>, respectively) (entries 1–3).

However, running the reaction in toluene gave a significantly different result (entries 4–7). First of all, conversions were all much higher than the equivalent experiments in THF (c.f. Table 2.2.7, entries 6–9), even with Na<sub>2</sub>CO<sub>3</sub> a conversion of 29% was achieved (see Table 2.2.14, entry 6). Full conversion was achieved with K<sub>3</sub>PO<sub>4</sub>, which gave only 36% conversion in THF with the same ligand, <sup>t</sup>Bu-MePhos (**2.41**). Steric properties of the ligand are therefore not the only factor dependent on the efficiency of the catalytic system.

**Table 2.2.14:** Solvent screening using the initial reaction.

| entry | solvent | base                            | conv. (%) <sup>a</sup> |
|-------|---------|---------------------------------|------------------------|
| 1     | dioxane | all four <sup>b</sup>           | <5                     |
| 2     | MeCN    | all four <sup>b</sup>           | <5                     |
| 3     | DMF     | all four <sup>b</sup>           | <5                     |
| 4     | toluene | Cs <sub>2</sub> CO <sub>3</sub> | 86                     |
| 5     | toluene | K <sub>3</sub> PO <sub>4</sub>  | >95                    |
| 6     | toluene | Na <sub>2</sub> CO <sub>3</sub> | 29                     |
| 7     | toluene | K <sub>2</sub> CO <sub>3</sub>  | 88                     |

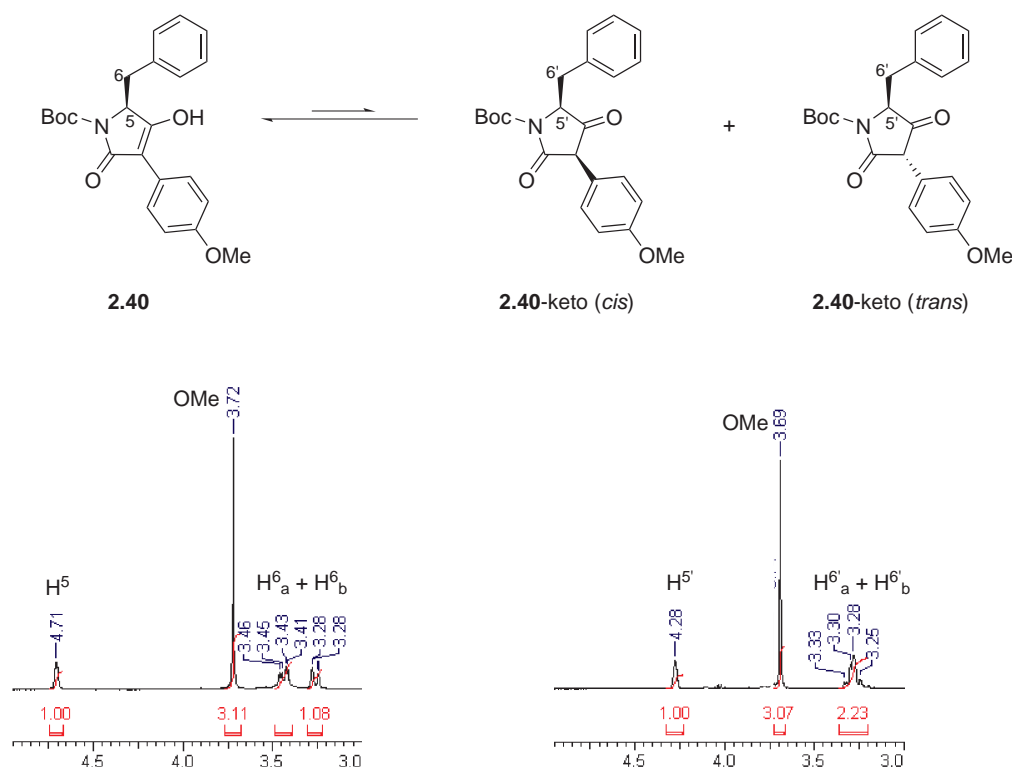
<sup>a</sup>: Determined by <sup>1</sup>H NMR. <sup>b</sup>: Tested with the four bases Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, and K<sub>2</sub>CO<sub>3</sub>, respectively in four different experiments.

### 2.2.3.6 Keto/enol equilibrium of $\alpha$ -aryl tetramic acids

In all cases, the crude product was isolated as the enol tautomer upon acidic work-up. However, during flash chromatography some degree of shifting in equilibrium towards the keto tautomer was discovered. That gave more complex NMR spectra and decreased solubility in organic solvents. To shift back the equilibrium, the compound was suspended in EtOAc and treated with 10% aqueous KHSO<sub>4</sub>, which subsequently dissolved the compound as the equilibrium was shifted toward the enol tautomer.

An example of this equilibrium shifting is shown in Figure 2.2.3 with the  $\alpha$ -aryl tetramic acid **2.40**. For both the enol and the keto tautomers, LC-MS showed only one peak with the same retention time. The acidic conditions on the LC-MS column apparently shift the equilibrium to one of the tautomers independent of the initial equilibrium position. However, the <sup>1</sup>H NMR spectrum of **2.40**-keto is more complex than the spectrum of **2.40**-enol because of broad peaks which might be a result of the coexistence of both a *cis*- and *trans*-tautomer. The chemical shift of H<sup>5</sup> is 4.71 ppm (dd) for the enol

tautomer, but 4.28 ppm (m) for the keto tautomers ( $H^{5'}$ ). The coupling pattern of the  $H^6$  diastereotopic protons also changes; for the enol-tautomer the two protons are two well-resolved double doublets ( $H^{6a} + H^{6b}$ ), whereas the keto tautomers show a multiplet in the region slightly upfield ( $H^{6'a} + H^{6'b}$ ). Based on  $^1H$  NMR it was not possible to determine the ratio between the *cis/trans* tautomers.



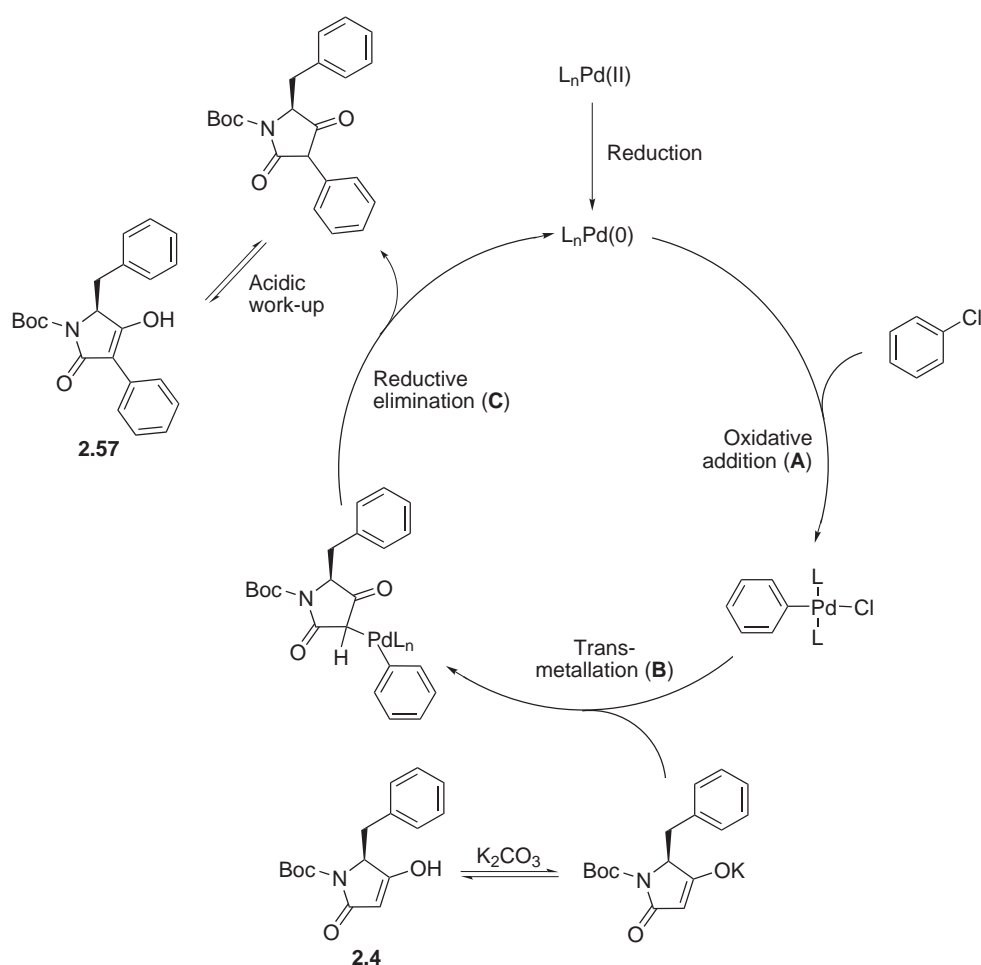
**Figure 2.2.3:** The observed tautomeric equilibrium and appurtenant  $^1H$  NMR spectra (upfield region); the spectrum to the left belongs to the enol tautomer of **2.40** and the spectrum to the right to the keto tautomers (*cis/trans*).

Surprisingly, the crude product isolated from the toluene/ $K_3PO_4$  reaction was exclusively the keto tautomer (**2.40-keto cis/trans**, see Figure 2.2.3), whereas THF gave the enol tautomer **2.40**, when the same acidic work-up procedure was used. To investigate this point further, the equilibrium of the enol tautomer **2.40** was tried to be shifted by dissolution in toluene, but nothing happened based on TLC analysis. When adding 10% aqueous  $KHSO_4$  the enol-tautomer was slowly shifted towards the keto tautomers (**2.40-keto cis/trans**). Isolation as the keto tautomers was unfortunately not always straightforward. It was found that it is easily shifted back to the enol tautomer. Formation of the less stable keto tautomers is not easy and it is rather unpredictable,

probably because the equilibrium position is dependent on concentration, temperature, pH and solvent. This corresponds with the literature regarding the keto-enol equilibrium of tetramic acids.<sup>199,201–203</sup>

### 2.2.3.7 Reaction mechanism

Based on the literature<sup>230</sup> the following mechanism for the coupling reaction is proposed (see Figure 2.2.4).



**Figure 2.2.4:** Proposed mechanism for the  $\alpha$ -arylation of tetramic acids.

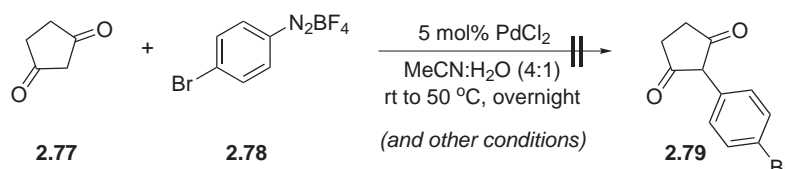
The catalytic cycle is assumed to be initiated by reduction of  $Pd(II)$  to the active  $Pd(0)$ , which might happen by a homo-coupling of the tetramic acids. Oxidation of the phosphine ligand is another well-known pathway for generation of  $Pd(0)$ . However, Barder

and Buchwald reported recently that dialkylbiaryl phosphines are highly resistant towards oxidation by molecular oxygen.<sup>311</sup>

Following reduction of Pd(II) to Pd(0), oxidative addition of the aryl halide takes place (**A**), then transmetalation by the potassium enolate of the tetramic acid **2.4** (**B**). Upon reductive elimination the product is released (**C**) and Pd(0) re-enters the catalytic cycle. The desired product **2.57** can be isolated by acidic work-up. It seems evident, that the position of the keto/enol equilibrium is not determined by the reaction mechanism, but rather by the work-up procedure and the solvent used.

### 2.2.3.8 Utilization of aryldiazonium salts

Based on the known reports on palladium-catalyzed cross-couplings utilizing an aryldiazonium salt,<sup>253,254,271</sup> it seems evident that these conditions also could be applicable for the  $\alpha$ -arylation of tetramic acids. Utilization of aryldiazonium salts as coupling partners would be a further broadening of utility and substrate scope. Hence, anilines could be used by *in situ* generation of the corresponding diazonium salt. It was therefore decided to briefly investigate the possibility to perform an  $\alpha$ -arylation on a 1,3-dicarbonyl system with an aryldiazonium salt, but also to implement the unusually mild reaction conditions typically reported for this type of chemistry.<sup>253,254,271</sup>

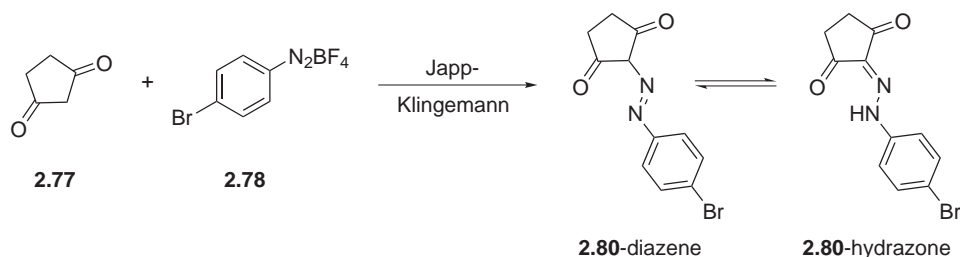


**Scheme 2.2.3:** Attempted cross-coupling of cyclopentane-1,3-dione (**2.77**) with the aryldiazonium salt **2.78**, but no product **2.79** was formed. Many screening conditions were tested, but they were all found to be unsuccessful.

Cyclopentane-1,3-dione **2.77** was selected as the model compound resembling the 1,3-dicarbonyl system of a tetramic acid. Inspired by Zaragoza<sup>271</sup> (c.f. Scheme 2.1.13), initial coupling conditions consisting of 5 mol% PdCl<sub>2</sub> in a solvent mixture of MeCN and H<sub>2</sub>O were chosen with the commercially available aryldiazonium tetrafluoroborate **2.78** (see Scheme 2.2.3).

Unfortunately, the product **2.79** was not formed with **2.78** as coupling partner. Only the corresponding hydrazone/diazone **2.80** was observed in minor to moderate quantities formed by addition of the nucleophilic  $\alpha$ -carbon to the electrophilic nitrogen of the

diazonium salt (see Scheme 2.2.4). The formation of **2.90** was observed on LC-MS and  $^1\text{H}$  NMR of the crude product. This reaction is known as the Japp-Klingemann reaction, which is usually promoted by strong base.<sup>267</sup>

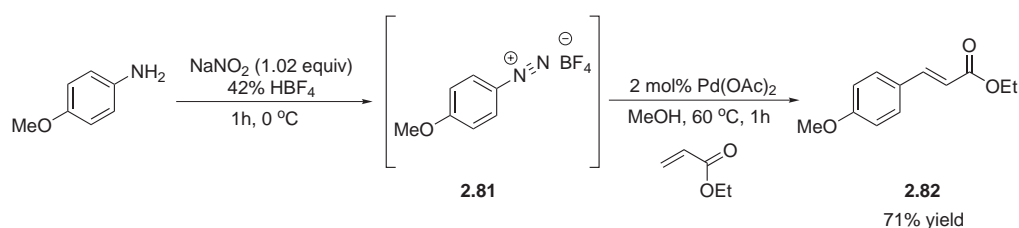


**Scheme 2.2.4:** Formation of hydrozone/diazenes **2.80** through direct nucleophilic attack of cyclopentane-1,3-dione (**2.77**) to the electrophilic diazonium salt **2.78**. This reaction is known as the Japp-Klingemann reaction.<sup>267</sup>

Furthermore, different temperatures were tested for the model reaction (see Scheme 2.2.3), but that did not afford **2.79**. Neither did addition of  $\text{H}_2\text{SO}_4$  (2 equiv) and/or  $\text{NaNO}_2$  (0.10 equiv), respectively, which would resemble reaction conditions for an *in situ*-generated aryl diazonium salt. Addition of acid would also prevent partial deprotonation of **2.77** and thereby potentially prevent the Japp-Klingemann reaction to proceed. Again, that was not observed, indicating that no palladium-catalyzed reaction took place. This was confirmed by running reactions in the absence of  $\text{PdCl}_2$  affording similar products as with the presence of the catalyst. Altering the order of addition was also tested, e.g. by addition of the aryl diazonium salt at last, but that did not change the reaction outcome.

Utilization of  $\text{PdCl}_2$  restricts the use of solvents, because a pure alcohol cannot be used. Predissolving  $\text{PdCl}_2$  in e.g. MeOH or EtOH did only result in 'palladium black' and not a clear, orange solution as was obtained by prestirring  $\text{PdCl}_2$  with MeCN overnight. A solvent mixture consisting of MeOH:MeCN: $\text{H}_2\text{O}$  (5:3:2) was successful for predissolution of  $\text{PdCl}_2$ , but did not afford any formation of the desired product **2.79**. 5 mol% Pd/C in MeOH was tested analogously to the results reported by Beller,<sup>255</sup> but only a messy crude product was obtained.

Sengupta and co-workers reported in 1993 a Heck cross-coupling utilizing 4-methoxybenzenediazonium tetrafluoroborate **2.81** and 2 mol% Pd(OAc)<sub>2</sub> in MeOH (see Scheme 2.2.5).<sup>312</sup> Inspired by these results, the same catalytical conditions were applied to the screening reaction (c.f. Scheme 2.2.3), but the altered catalyst, solvent and coupling partner did not give successful results. Addition of H<sub>2</sub>SO<sub>4</sub> (1.0 equiv) or K<sub>3</sub>PO<sub>4</sub> (1.0 equiv), respectively, was not successful.



**Scheme 2.2.5:** Palladium-catalyzed Heck cross-coupling utilizing *in situ*-generated aryldiazonium tetrafluoroborate **2.81** affording the ethyl ester **2.82** in good yield.

So far, no coupling was observed with aryldiazonium tetrafluoroborates (**2.78** or **2.81**) and the cyclopentane-1,3-dione (**2.77**) as a model substrate. To investigate if the unreactivity was a result of decomposed catalysts or a pronounced unreactivity of the tetrafluoroborates (since these salts are significantly more stable than other diazonium salts), a range of reference reactions were performed. Basically, the results published by Beller,<sup>255</sup> Zaragoza<sup>271</sup> and Sengupta<sup>312</sup> were easily reproduced and the cross-couplings proceeded as described in the literature. These results indicated that all three catalysts (Pd/C, PdCl<sub>2</sub> and Pd(OAc)<sub>2</sub>) were not decomposed on the shelf and still active under the tested conditions. Furthermore, the results of Sengupta and co-workers<sup>312</sup> were tested with both *in situ*-generated and commercially available aryldiazonium salt **2.81**. Both reactions proceeded nicely, in fact, a better yield was obtained with commercially available **2.81** (83%). Thus, NaNO<sub>2</sub> and HBF<sub>4</sub> were not required for a successful palladium-catalyzed coupling.

One plausible explanation for the unreactivity seen so far of the cyclopentane-1,3-dione (**2.77**) might be that this chemistry works much better with electron-poor alkenes analogously to the Heck reaction. Furthermore, the reactivity and solubility of the aryldiazonium salt might also influence the overall outcome. For example the reaction conditions reported by Zaragoza<sup>271</sup> were tested with 4-bromobenzenediazonium tetrafluoroborate **2.78** and allyl alcohol, but no reaction took place. Apparently, this reaction requires a much more reactive coupling partner. Finally, solubility of aryldiazonium salts is low in THF, toluene and MeCN, which are usually successful solvents for cross-couplings. Con-

trarily, aryldiazonium salts are soluble in mixtures of MeCN/H<sub>2</sub>O as well as in MeOH, EtOH, DMF and DMSO. These solubilities were tested for 4-bromobenzenediazonium tetrafluoroborate **2.78**.

Selection of catalyst may also be crucial, especially if a source of Pd(II) is used, because reduction to Pd(0) is required for reactivity. This activation of the catalyst may take place e.g. by homo-coupling or oxidation of an added ligand. Phosphines (e.g. (PPh<sub>3</sub>)<sub>3</sub>), if added, may add to the electrophilic nitrogen of aryldiazonium salt followed by a decomposition and release of N<sub>2</sub>, thus forming a cationic arylpalladium complex,<sup>313</sup> which might undergo a cross-coupling. On the other hand, phosphines are also known to transfer an electron to the aryldiazonium salt affording an aryl radical, which ultimately gives the reduction product Ar-H and phosphine oxide.<sup>253</sup> This explains why phosphines are not ligands of choice in palladium-catalyzed reactions with aryldiazonium salts.

Based on these disappointing results, implementation of aryldiazonium salts in the  $\alpha$ -arylation of tetramic acids was discontinued. Apparently, 1,3-dicarbonyl substrates are not suitable for a ligand-free cross-coupling catalyzed by PdCl<sub>2</sub>, Pd/C or Pd(OAc) in MeCN:H<sub>2</sub>O. Yet, these reaction conditions are rather thankless compared to traditional cross-coupling conditions. Implementation of the aryldiazonium salt in the previously developed  $\alpha$ -arylation of tetramic acids was not tested, primarily because of its little impact if the remarkable mild reaction conditions could not also be used.

### 2.2.3.9 Summary

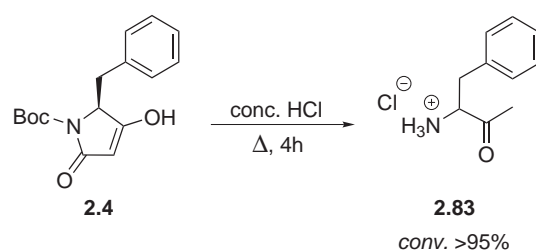
A new, mild, and racemization-free palladium-catalyzed  $\alpha$ -arylation of tetramic acids has been developed giving rise to  $\alpha$ -aryl phenylalanine-derived tetramic acids (**2.40** and **2.57–2.70**) in good yield (60–79%). Through optimization it was found that 2 mol% Pd(OAc)<sub>2</sub> and 4 mol% *t*Bu-XPhos (**2.44**) gave full conversion in THF at 80 °C after 1 hour for most substrates. The two weak inorganic potassium bases, K<sub>2</sub>CO<sub>3</sub> and K<sub>3</sub>PO<sub>4</sub>, worked equally well. *t*Bu-MePhos (**2.41**), which is a less sterically hindered ligand, gave full conversion with K<sub>3</sub>PO<sub>4</sub> in toluene. A variety of different substrates was tested and a wide range of functionalities was tolerated, e.g. ethers, esters, ketones, alcohols, nitriles and nitro groups. With respect to the substitution pattern of the aryl chloride, electron-withdrawing as well as electron-donating groups showed similar reactivity and *meta*- and *para*-substituted aryl chlorides reacted identically. Due to steric hindrance *ortho*-substituted aryl chlorides did not react. Aryl chlorides, bromides and triflates all coupled nicely, whereas aryl iodides and tosylates did not work. The  $\alpha$ -arylation can be facilitated by microwave heating with reaction time down to 5 minutes at 110 °C. Functionalized amino acid-derived tetramic acids were also applicable for this chemistry, giving rise to e.g. tyrosine- (**2.72**), lysine- (**2.73**) and more importantly the arginine-derived (**2.71**)



$\alpha$ -aryl tetramic acid in good yield (67–69%). Heterocycles and unprotected amines were not compatible with the chemistry. Utilization of aryldiazonium tetrafluoroborates as coupling partners for the  $\alpha$ -arylation of a 1,3-dicarbonyl model compound was not found to be useful with either  $\text{Pd}(\text{OAc})_2$ ,  $\text{PdCl}_2$  or  $\text{Pd/C}$  in  $\text{MeCN}/\text{H}_2\text{O}$  or  $\text{MeOH}$ .

## 2.2.4 Cleavage of $\alpha$ -aryl tetramic acids

The most appropriate approach for cleavage of the  $\alpha$ -aryl tetramic acids would be a one-step procedure, which does not require a reduction of the ketone functionality prior to ring opening. Initially the opening of  $\text{Boc-pyPhe-OH}$  (**2.4**) was attempted with concentrated aqueous  $\text{NaOH}$  or concentrated  $\text{HCl}$ , respectively.  $\text{NaOH}$  did not give any ring opening, probably because of salt formation. Concentrated  $\text{HCl}$  on the other hand, led to ring cleavage upon heating to reflux for 4 hours (see Scheme 2.2.6).

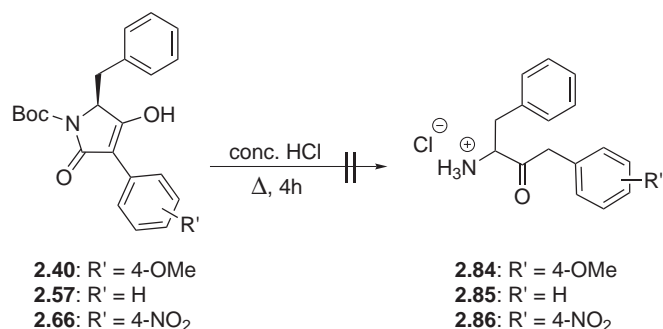


**Scheme 2.2.6:** *Boc-pyPhe-OH* (**2.4**) was cleaved to the corresponding methyl ketone **2.83** by treatment with concentrated  $\text{HCl}$  at reflux for 4 hours.

The corresponding methyl ketone **2.83** was achieved in full conversion (>95%). No ring cleavage was afforded by treatment with concentrated  $\text{HCl}$  at room temperature, not even with reaction times as long as several days. Analogously,  $\text{Boc-pyArg}(\text{Pbf})\text{-OH}$  (**2.32**),  $\text{Boc-pyLys}(\text{Cbz})\text{-OH}$  (**2.34**) and  $\text{Boc-pyOrn}(\text{Cbz})\text{-OH}$  (**2.38**), respectively, were subjected to concentrated  $\text{HCl}$  at reflux revealing similar reactivity of **2.32** and **2.34** (Boc- and Pbf-protections were lost). Ring opening was, on the other hand not obtained by treating  $\text{Boc-pyOrn}(\text{Cbz})\text{-OH}$  (**2.38**) with concentrated  $\text{HCl}$  which led to messy crude products.

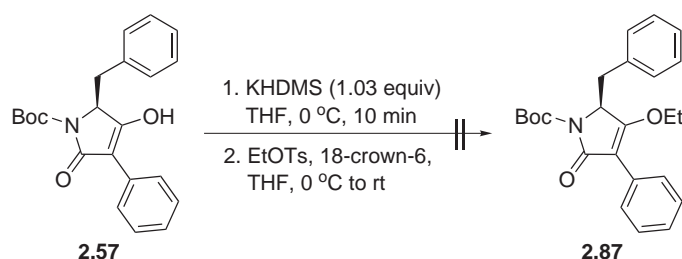
To test if these promising conditions also could cleave  $\alpha$ -aryl tetramic acids, the previously synthesized compounds **2.40**, **2.57** and **2.66** were subjected to concentrated  $\text{HCl}$  at reflux for 4 hours (see Scheme 2.2.7).

Unfortunately, no ring cleavage was observed indicating that  $\alpha$ -aryl tetramic acids are much more stable than the parent tetramic acids. Prolonged reaction times did still



**Scheme 2.2.7:** Attempted ring cleavage of the  $\alpha$ -aryl tetramic acids **2.40**, **2.57** and **2.66** with concentrated HCl was not successful.

not give any traces of product. Ring opening would probably be possible under basic conditions, if the acidic proton of the  $\alpha$ -aryl tetramic acid was removed. This would avoid salt formation and the ring system might be cleaved leading to the corresponding  $\gamma$ -amino- $\alpha$ -aryl- $\beta$ -keto carboxylate.

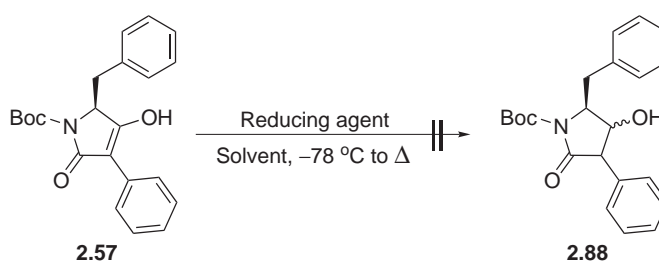


**Scheme 2.2.8:** Conversion of the  $\alpha$ -aryl tetramic acid **2.57** into the corresponding ethyl enol ether **2.87** did not work.

It was therefore attempted to convert the  $\alpha$ -aryl tetramic acid **2.57** into the corresponding ethyl enol ether **2.87** (see Scheme 2.2.8), inspired by a similar reaction developed for tetramic acids.<sup>212</sup> Formation of an alkyl enol ether would remove the acidity of the tetramic acid and prevent salt formation upon treatment with base. Unfortunately **2.57** was unreactive under these reaction conditions.

One reason for this increased stability and unreactivity might be the enhanced conjugated system present in the  $\alpha$ -aryl tetramic acids. To reduce this conjugation and to decrease the acidity, reduction of the ketone functionality of **2.57**, forming the  $\beta$ -hydroxy derivative **2.88** was attempted with a variety of reducing agents; NaBH<sub>4</sub>, LiBH<sub>4</sub>, DIBAL-H, L-selectride, LiAl(O<sup>t</sup>Bu)<sub>3</sub>H and 9-BBN (see Scheme 2.2.9). Unfortunately, for all of

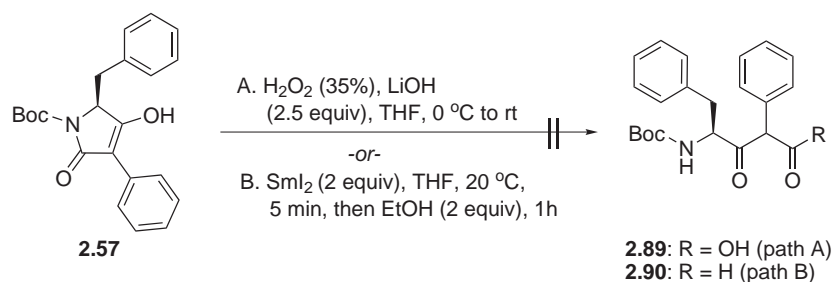
them only the starting material **2.57** was isolated upon work-up. Different temperatures were tested, ranging from  $-78\text{ }^{\circ}\text{C}$  to reflux as well as different equivalents of the reducing agent, but no change in the outcome was observed. Addition of AcOH did not lead to product formation. Some of the reducing agents were also tested with the  $\alpha$ -aryl tetramic acids **2.40** and **2.66**, but no change in reactivity was observed despite the altered electrondensity of the aryl group.



**Scheme 2.2.9:** The  $\alpha$ -aryl tetramic acid **2.57** was attempted to be reduced to **2.88** with a variety of different reducing agents ( $\text{NaBH}_4$ ,  $\text{LiBH}_4$ , DIBAL-H, *L*-selectride,  $\text{LiAl}(\text{O}^t\text{Bu})_3\text{H}$  and 9-BBN), but none of them worked.

So far, none of the approaches for ring cleavage, reduction of the ketone functionality or ethyl enol ether formation afforded the desired product. It was therefore decided to change strategy radically.  $\text{LiOOH}$  (path A)<sup>278</sup> and  $\text{Sml}_2$  (path B),<sup>284</sup> respectively, were tested (see Scheme 2.2.10).

Both of these methods represent mechanistically different approaches for ring cleavage compared to the methods previously tested. Neither of the methods gave any product formation, **2.89** or **2.90**, respectively.



**Scheme 2.2.10:** Neither  $\text{LiOOH}$  (path A) nor  $\text{Sml}_2$  (path B) afforded any cleavage of the  $\alpha$ -aryl tetramic acid **2.57** to the corresponding carboxylic acid **2.89** and the aldehyde **2.90**, respectively.

#### 2.2.4.1 Summary

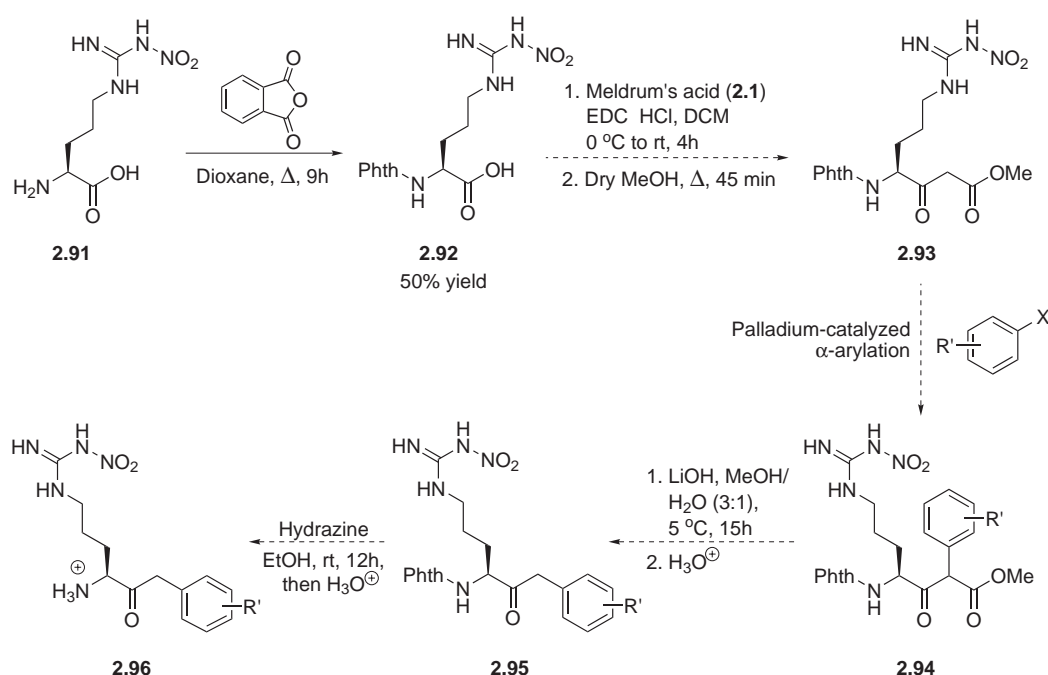
Ring opening of the synthesized  $\alpha$ -aryl tetramic acids did not work. Apparently, these compounds are much more stable than the parent tetramic acids, and hence unreactive upon treatment with concentrated  $\text{HCl}$  or with reducing agents such as  $\text{NaBH}_4$ ,  $\text{LiBH}_4$ , DIBAL-H, L-Selectride,  $\text{LiAl}(\text{O}^t\text{Bu})_3\text{H}$  or 9-BBN. Treatment with  $\text{LiOOH}$  or  $\text{Sml}_2$ , respectively, did not work either. Ethyl enol ether derivatization for subsequent basic ring opening was not successful, once again due to the unreactivity of the  $\alpha$ -aryl tetramic acids.

## 2.3 Conclusion

Optimization of the facile synthesis of  $\gamma$ -amino- $\beta$ -keto esters failed due to formation of the corresponding tetramic acid. To avoid this cyclization, Boc<sub>2</sub>-Phe-OH (**2.18**) was synthesized in three steps from the parent Boc-Phe-OH (**2.2**) in excellent yield with no racemization. This diprotected amino acid was subjected to the reaction conditions and a variety of different  $\gamma$ -amino- $\beta$ -keto esters **2.21–2.24** were synthesized in high yield. Unfortunately, this strategy was inconvenient for functionalized amino acids due to lack of selectivity among the *N*<sup>α</sup>- and the side-chain nitrogens resulting in many undefined products. The tetramic acids were therefore selected as the key intermediate of choice for further synthetic development. Tetramic acids were easily synthesized in high yield from the corresponding amino acids and Meldrum's acid (**2.1**) with no by-product formation. A palladium-catalyzed  $\alpha$ -arylation of the tetramic acids was then developed by careful screening of multiple reaction parameters such as different ligands, bases, coupling partners and solvents. The most appropriate conditions were 2 mol% Pd(OAc)<sub>2</sub> and 4 mol% *t*Bu-XPhos (**2.44**) in THF (80 °C, 1–16h) which afforded a broad range of  $\alpha$ -aryl tetramic acids **2.40**, **2.57–2.70** showing a very good functional group tolerance. Moreover, arylchlorides and aryltriflates showed similar reactivity compared to arylbromides. The chemistry was also applicable with microwave heating at 110 °C for 5 minutes. Aryldiazonium tetrafluoroborates were not found to be suitable for  $\alpha$ -arylation of 1,3-dicarbonyl compounds. The  $\alpha$ -aryl tetramic acids were subjected to many different approaches for ring opening toward to the synthesis of the desired benzyl ketone building block, but that did not succeed. Apparently, these arylated compounds are much more stable than the parent tetramic acids. Hydrolysis with concentrated HCl, reduction with a variety of different reducing agents and treatment with LiOOH or Sml<sub>2</sub>, respectively, did only afford recovery of the starting material. Based on the surprising unreactivity of  $\alpha$ -aryl tetramic acids, development of a new, mild and convenient building block synthesis was not successful.

## 2.4 Outlook

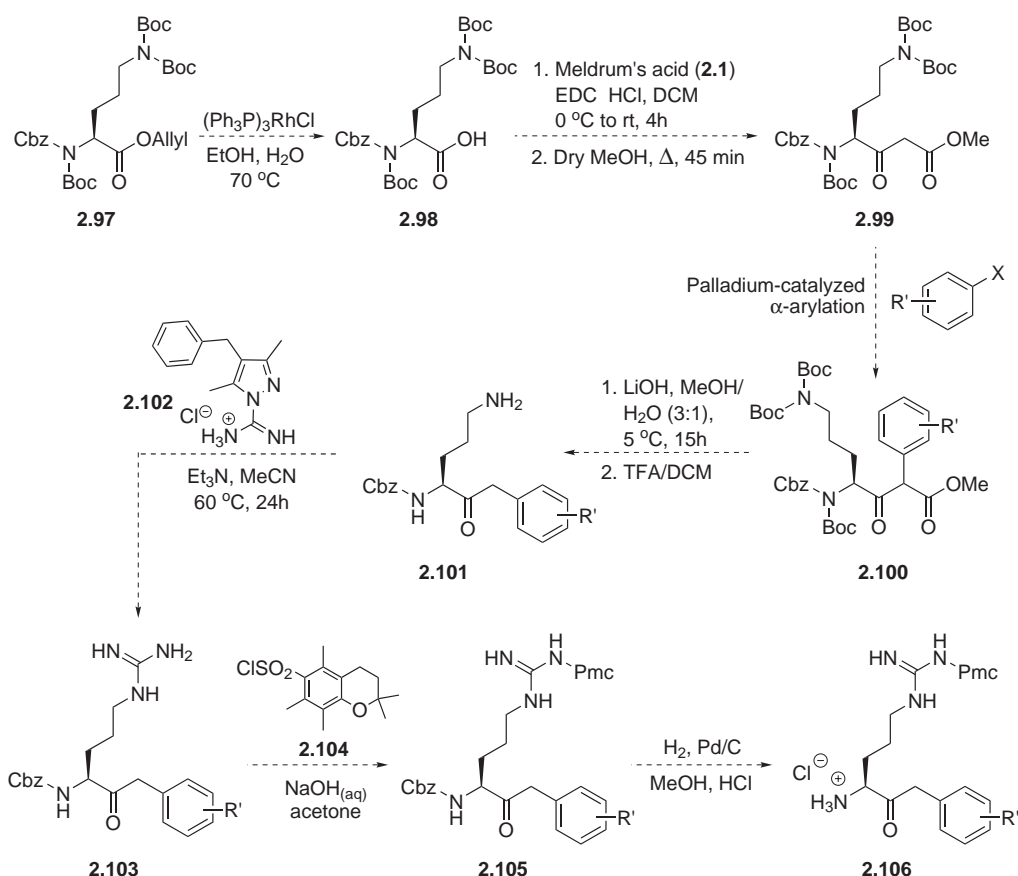
Despite the many attempts to ring open the  $\alpha$ -aryl tetramic acids, this strategy seems to be useless due to the pronounced unreactivity. Going back to the other key intermediate, the  $\gamma$ -amino- $\beta$ -keto esters, these compounds might be useful if another diprotection strategy is considered. Some phthaloyl-protected amino acids are commercially available,<sup>251,314</sup> but arginine-derived compounds are not. This protecting group therefore has to be introduced prior to synthesis of the  $\beta$ -keto ester (see Scheme 2.4.1).



**Scheme 2.4.1:** Proposed synthesis of the arginyl benzyl ketone building block **2.96** by phthaloyl *N,N*-protection to avoid tetramic acid formation during  $\beta$ -keto ester synthesis.

*N,N*-Protection of H-Arg(NO<sub>2</sub>)-OH (**2.91**) has been reported in the literature using phthalic anhydride giving Phth-Arg(NO<sub>2</sub>)-OH (**2.92**) in modest yield.<sup>315</sup> However, this reaction was reported to be selective and only the amino group was protected. This selectivity is important in contrast to the non-selective introduction of an additional Boc-group, which was found inconvenient for functionalized amino acids such as arginine. The diprotected amino acid **2.92** is then thought to be converted into the corresponding  $\beta$ -keto methyl ester **2.93** by *C*-acylation of Meldrum's acid (**2.1**) and work-up with MeOH. Due to the fully protected amino-group, no tetramic acid will be formed

by cyclization.<sup>174</sup> Using appropriate reaction conditions, the aryl-group could be introduced by a palladium-catalyzed reaction. Unfortunately, this may require another careful screening of possible ligands, solvents and bases if the previously reported conditions for  $\alpha$ -arylation of tetramic acids cannot be used. Followed by basic hydrolysis of the methyl ester with LiOH in MeOH/H<sub>2</sub>O<sup>316</sup> giving the protected arginyl ketone building block **2.95**, the desired building block **2.96** is believed to be prepared by *N,N*-deprotection by treatment with hydrazine.<sup>294,295,317</sup> The -NO<sub>2</sub> guanine protection can be removed after peptide synthesis by reduction with H<sub>2</sub> over Pd/C.<sup>318</sup> Utilization of the highly toxic hydrazine is a major drawback of this strategy as well as the lack of commercially available starting materials and the use of non-standardized amino acid protection groups. However, it might represent a possible synthetic route for functionalized benzyl ketone building blocks.



**Scheme 2.4.2:** Proposed synthesis of the arginyl benzyl ketone building block **2.106** via ornithine, which after  $\beta$ -keto ester formation and  $\alpha$ -arylation is amidated and Pmc-protected.

To avoid the nucleophilic behaviour of the guanine group, an arginine precursor could be used instead. The non-DNA encoded amino acid ornithine (Orn) is an obvious precursor of arginine. Cbz-Orn-OH is thought to be converted to Cbz-Orn-OAllyl, and then to (Boc,Cbz)-Orn(Boc)<sub>2</sub>-OAllyl (**2.97**), analogously with preparation of Boc<sub>2</sub>-Orn(Cbz)-OAllyl (**2.30**) described in Section 2.2.1.4. The reverse order of the Cbz group (*N*<sup>α</sup> versus *N*<sup>δ</sup>) is chosen to secure regioselective deprotection for amidination of the δ-amino group only. Removal of the allyl group could be done by treating **2.97** with (Ph<sub>3</sub>P)<sub>3</sub>RhCl affording the carboxylic acid **2.98**,<sup>319</sup> which is then converted to the γ-amino-β-keto methyl ester **2.99** as previously described (see Scheme 2.4.2). The aryl-group is introduced by a palladium-catalyzed cross-coupling giving **2.100**, which is then hydrolyzed to the β-keto acid following a decarboxylation upon treatment with TFA which is thought to give the amine **2.101**. Treatment with TFA promotes the decarboxylation but also removes all the Boc-groups. The free δ-amino group is then amidinated using 4-benzyl-3,5-dimethyl-1*H*-pyrazole-1-carboxamide hydrochloride (**2.102**) affording the unprotected arginine **2.103**.<sup>320</sup> The amidinating agent **2.102** can be synthesized easily in two steps from benzaldehyde, acetylacetone and aminoguanidine hydrochloride.<sup>320</sup> Introduction of the *N*<sup>ω</sup>-Pmc protection is thought to be accomplished by reaction with the commercially available Pmc-Cl **2.104** under basic conditions,<sup>321</sup> and the final preparation of the arginyl benzyl ketone building block **2.106** is achieved by removal of the Cbz-group upon catalytic hydrogenolysis.<sup>322</sup>

Both of these alternative strategies are somewhat tedious because of the many protecting group manipulations. However, they might also represent possible routes to functionalized benzyl ketones for probing of the S1' binding pocket of FVIIa, which could otherwise not be introduced. Probing of the S1' site represents an important strategy for designing of potent and selective serine protease inhibitors - a strategy that is almost unexplored.



## 2.5 Experimental

### General procedures

Chemicals and reagents were all commercially available and used without further purification. Solvents were of HPLC quality and used without further purification. THF and DMSO-*d*<sub>6</sub> were dried over 3Å molecular sieves (1.6 mm pellets) prior to use, whereas alcohols for  $\gamma$ -amino- $\beta$ -keto ester synthesis were dried over 4Å molecular sieves. Evaporation *in vacuo* was performed on Büchi Vacuum Rotavapor R-205 at approx. 40 °C and down to approx. 20 mbar. Microwave heating was achieved in a Biotage<sup>®</sup> Initiator<sup>TM</sup> Microwave Synthesizer (single-mode) set with high absorption. The reaction was performed in a Biotage<sup>®</sup> 10 mL microwave vial sealed with a septum cap, and prestirred in the synthesizer for 1 minute and then heated to 110 °C, after which the reaction was stirred for 5 minutes. Finally, the vial was cooled to below 50 °C with air over a few minutes. During the reaction both temperature (IR-sensor) and pressure (bowing of the septum) were monitored automatically by the instrument. TLC was performed using Merck Silica gel 60 F<sub>254</sub> aluminum sheets. The plates were visualized in UV light (254 nm), stained with either 0.5% ninhydrin in EtOH, 5% MoO<sub>3</sub>/H<sub>3</sub>PO<sub>4</sub> in EtOH or 1.5% KMnO<sub>4</sub> in conc. H<sub>2</sub>SO<sub>4</sub>, respectively, and subsequently heated with a heating gun. Flash chromatography was performed on a Biotage<sup>®</sup> FlashMaster purification system. The eluent ratios given are vol%. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were recorded on a Bruker Avance DRX 400 instrument using deuterated solvents as internal references. Chemical shifts ( $\delta$ ) are given in ppm and coupling constants (*J*) in Hertz. HRMS was performed on a Agilent Technologies 1200 Series LC/MSD-TOF instrument using a C18 column (5%→95% MeCH in H<sub>2</sub>O, 15 minutes). The found mass (*m/z*) was stated along with the appurtenant fragment, which varies less than 5 ppm compared to the calculated exact mass. MS was determined on a Perkin Elmer 200 Series PE Sciex API 3000 LC/MS/MS Turbo Ionspray system (5%→90% MeCH in H<sub>2</sub>O, 10 minutes). Elemental analysis was performed by Micro Kemi AB, Sweden and varied less than 0.4% compared to the calculated values. In some instances elemental analysis varied more than 0.4% compared to the calculated composition, even though analytical HPLC showed a pure compound. Melting points were measured on a Büchi 535 Melting Point Apparatus and given in degrees Celsius (°C), uncorrected. Analytical UPLC (TFA) was performed on a Waters Acquity Ultra Performance LC using a gradient of 5%→95% (or variations hereof) MeCH in H<sub>2</sub>O (16 minutes) containing 0.05% TFA on a C18 column (1.7  $\mu$ m, 2.1×50 mm). IR analysis was carried out on a Bruker Alpha FT-IR spectrometer using attenuated total reflection (ATR) sampling technique. Only the strongest or structurally most significant peaks are included in the experimental section, stated in cm<sup>-1</sup>. Chiral HPLC was run on an Agilent 1100 series instrument with an AS-H column (amylose tris[(*S*)- $\alpha$ -methylbenzyl-carbamate] coated on 5  $\mu$ m silica gel), L = 250 mm, I.D. = 4.6 mm. 15 vol% EtOH in hexanes was used as eluent (isocratic); flow rate was 1.00 mL/min, max. 70 bars and a run time of 15 minutes. 5  $\mu$ L sample

was injected from a solution of the compound in 15 vol% EtOH in hexanes (sample concentration: 1 mg/mL). Enantiomeric excess was calculated using the average of the peak integrals at the following wavelengths: 210, 222, 230, 254 and 280 nm, respectively.

### General synthesis of tetramic acids (A)

To a solution of a *N*-Boc amino acid (12.5 mmol, 1.0 equiv) in DCM (165 mL), Meldrum's acid (**2.1**) (2.70 g, 18.8 mmol, 1.5 equiv) and DMAP (2.29 g, 18.8 mmol, 1.5 equiv) were added. The mixture was cooled to 0 °C and EDC·HCl (3.60 g, 18.8 mmol, 1.5 equiv) was added while stirring. Stirring was continued at 0 °C for 15 minutes and then at room temperature for 4 hours. The yellow mixture was transferred to a separatory funnel with EtOAc (490 mL) and washed with brine (2 × 250 mL), 5% aqueous citric acid (2 × 250 mL) and finally with brine again (250 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. EtOAc (100 mL) was added and the mixture was heated to reflux for 30 minutes. The solution was cooled to ambient temperature and the solvent was removed by evaporation *in vacuo* and the residue was coevaporated with toluene (3 × 50 mL) and dried in high vacuum overnight. This afforded the desired tetramic acid, which was used without purification.

### (5S)-5-Benzyl-1-(*tert*-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-*py*Phe-OH) (**2.4**)<sup>171</sup>

Following the general synthesis of tetramic acids (A) using Boc-Phe-OH (**2.2**) as amino acid starting material, afforded the product as a pale, yellow solid (3.48 g, 96%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 12.41 (broad s, 1H), 7.27–7.19 (m, 3H), 7.01–6.98 (m, 2H), 4.65 (s, 1H), 4.62 (dd, *J* = 5.3, 2.6 Hz, 1H), 3.35 (dd, *J* = 13.9, 5.3 Hz, 1H), 3.06 (dd, *J* = 13.9, 2.3 Hz, 1H), 1.50 (s, 9H). HRMS (*m/z*) calcd. for C<sub>32</sub>H<sub>38</sub>N<sub>2</sub>O<sub>8</sub>Na [2M + Na]<sup>+</sup>: 601.2520, found: 601.2516. HPLC purity (TFA): >92.2%. Mp ≈ 154 °C (decomp.) (lit.:<sup>171</sup> 120–124 °C).

### General synthesis of benzyl/allyl amino acid-derived esters (B)

A *N*-Boc protected amino acid (1.42 mmol, 1.00 equiv) was dissolved in MeOH (10 mL) and H<sub>2</sub>O (1.5 mL) was added. The solution was neutralized to pH 7 with 20% aqueous Cs<sub>2</sub>CO<sub>3</sub> (30 drops). The mixture was evaporated to dryness *in vacuo* and the residue was coevaporated with DMF (2 × 25 mL). The cesium salt was stirred with BnBr (300 μL, 292 mg, 1.71 mmol, 1.20 equiv) or allylbromide (150 μL, 189 mg, 1.56 mmol, 1.10 equiv) in DMF (10 mL) at room temperature for 40 min (BnBr) or 2 hours (allylbromide). The reaction mixture was transferred to a separatory funnel with EtOAc (60 mL) and extracted with brine (2 × 60 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. The product was used without further purification.

**(2S)-Benzyl 2-(tert-butoxycarbonyl)amino-3-phenylpropanoate (Boc-Phe-OBn) (2.17).**<sup>306</sup>

Following the general synthesis of benzyl carboxylic-protected amino acids (**B**) with Boc-Phe-OH (**2.2**) and BnBr (5.9 mL, 49.76 mmol, 1.1 equiv) as starting materials, afforded the product as a slightly yellow solid (15.64 g, 97%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>),  $\delta$  7.27–7.12 (m, 8H), 6.95–6.93 (m, 2H), 5.07 (d,  $J$  = 12.3, 1H), 5.00 (d,  $J$  = 12.2, 1H), 4.90 (d,  $J$  = 8.2, 1H), 4.53 (dd,  $J$  = 14.2, 6.0, 1H), 3.05–2.92 (m, 2H), 1.31 (s, 9H). HRMS ( $m/z$ ) calcd. for C<sub>21</sub>H<sub>25</sub>NO<sub>4</sub>Na [M + Na]<sup>+</sup>: 378.1676, found: 378.1671. Mp 62–63 °C (lit.:<sup>306</sup> 64–65 °C).

**General synthesis of N,N-Boc<sub>2</sub>-protected amino acids (C)**

To a solution of a benzyl/allyl *N*-Boc amino acid-derived ester (45.23 mmol, 1.0 equiv) in MeCN (60 mL) was added DMAP (0.55 g, 4.52 mmol, 0.1 equiv) and Boc<sub>2</sub>O (10.86 g, 49.76 mmol, 1.1 equiv). The reaction was stirred at room temperature overnight. Additional Boc<sub>2</sub>O ( $\approx$  5 g, 24.88 mmol, 0.51 equiv) was added repeatedly until TLC and/or LC-MS showed full conversion of the starting material. The mixture was evaporated to dryness *in vacuo* and then transferred to a separatory funnel with EtOAc (150 mL). Washed with 5% aqueous citric acid solution (150 mL) and brine (150 mL). The yellow organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, evaporated to dryness *in vacuo* and the residue was coevaporated with toluene (3  $\times$  150 mL) and dried in high vacuum. The product was used without further purification.

Carboxylic deprotection was achieved by dissolving the *N,N*-Boc<sub>2</sub> protected benzyl ester in MeOH (2.5 mL/g) adding 5 wt.% Pd/C. Hydrogenated with H<sub>2</sub> gas applied by a balloon (1 atm) at room temperature and stirred vigorously overnight. The catalyst was removed by filtration through Celite<sup>®</sup> and rinsed with MeOH (3  $\times$  10 mL). The filtrates were evaporated to dryness *in vacuo* and dried in high vacuum. Deprotection of the allyl esters was never tried.

Enantiomeric excess was determined by derivatization with (*R*)-1-phenyl ethylamine: a carboxylic acid sample (0.20 mmol, 1.00 equiv) was dissolved in DCM (4 mL), which was added HOBt (40 mg, 0.30 mmol, 1.50 equiv) and EDC·HCl (29 mg, 0.24 mmol, 1.20 equiv). After stirring at ambient temperature for 5 minutes, DIPEA (170  $\mu$ L) was added and the reaction mixture was stirred overnight securing full conversion (>95%). Then EtOAc (15 mL) was added and the mixture was washed with H<sub>2</sub>O (25 mL), brine (25 mL), 0.5M HCl (25 mL) and finally with brine again (25 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*, which gave the desired derivative. The enantiomeric excess was determined by <sup>1</sup>H NMR analysis in CDCl<sub>3</sub> using the chemical shifts for the methyl group. A reference with racemic carboxylic acid sample was run to determine the shifts of the methyl groups in both diastereomers.

**(2S)-2,2-di(tert-Butoxycarbonyl)amino-3-phenylpropanoic acid (Boc<sub>2</sub>-Phe-OH) (2.18)**<sup>306,323</sup>

Following the general synthesis of *N,N*-Boc<sub>2</sub>-protected amino acids (**C**) with Boc-Phe-OBn (**2.17**) as starting material, afforded Boc<sub>2</sub>-Phe-OBn as a viscous orange oil (19.02 g, 92%). <sup>1</sup>H NMR (CDCl<sub>3</sub>), 7.36–7.16 (m, 10H), 5.24–5.15 (m, 3H), 3.45 (dd, *J* = 15.0, 6.0, 1H), 3.24 (dd, *J* = 15.0, 12.0, 1H), 1.35 (s, 18H). Upon hydrogenolysis of the benzyl ester, the crude product crystallized spontaneously to a pale, yellow solid (11.57 g, 70%). Smaller scale gave up to 90% yield. Purification was achieved by recrystallization from toluene (2 mL/g crude product), which afforded **2.18** as white crystals (81% recovery from recrystallization). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.31–7.17 (m, 5H), 5.24 (dd, *J* = 9.0, 6.0, 1H), 3.43 (dd, *J* = 12.0, 3.0, 1H), 3.24 (dd, *J* = 15.0, 9.0, 1H), 1.40 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 176.3, 151.6, 137.3, 129.5, 128.4, 126.6, 83.3, 59.2, 35.8, 27.8. HRMS (*m/z*) calcd. for C<sub>38</sub>H<sub>54</sub>N<sub>2</sub>O<sub>12</sub>Na [2M + Na]<sup>+</sup>: 753.3568, found: 753.3583. Elem. anal. calcd. for C<sub>19</sub>H<sub>27</sub>NO<sub>6</sub>: C 62.45, H 7.45, N 3.83, found: C 62.77, H 7.53, N 3.74. Mp 142–144 °C (litt.: 137–138 °C). IR (KBr): ν 2987, 1723 (strong), 1371, 1318, 1249, 1121, 1053, 847, 783, 703 cm<sup>-1</sup>. The enantiomeric excess was determined to be >95% (diagnostic chemical shift: δ 1.49 (d, *J* = 7.16 Hz, 3H), diastereomeric reference: δ 1.47 (d, *J* = 6.78 Hz, 3H) was not observed).

**(2S)-Methyl 2,2-di(tert-butoxycarbonyl)amino-3-phenylpropanoate (Boc<sub>2</sub>-Phe-OMe) (2.20)**<sup>324</sup>

Following the general synthesis of *N,N*-Boc<sub>2</sub>-protected amino acids (**C**) (except the second step, the hydrogenolysis) with Boc-Phe-OMe (**2.19**) as starting material, afforded the product as an oil (1.32 g, 97%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.30–7.16 (m, 5H), 5.15 (dd, *J* = 10.2, 4.9 Hz, 1H), 3.75 (s, 3H), 3.43 (dd, *J* = 14.1, 5.1 Hz, 1H), 3.21 (dd, *J* = 13.9, 10.6 Hz, 1H), 1.38 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 170.9, 151.6, 137.6, 129.5, 128.3, 126.5, 82.9, 59.4, 52.2, 36.2, 27.8. MS (*m/z*) calcd. for C<sub>20</sub>H<sub>29</sub>NO<sub>6</sub>Na [M + Na]<sup>+</sup>: 402.2, found: 402.1.

**Attempted basic hydrolysis of Boc<sub>2</sub>-Phe-OMe (2.20) for preparation of enantiomerically pure Boc<sub>2</sub>-Phe-OH (2.18)**

Boc<sub>2</sub>-Phe-OMe (**2.20**) (200 mg, 0.53 mmol, 1.00 equiv) was dissolved in MeOH (or THF) (7.5 mL) and cooled to 5 °C (other experiments were performed at room temperature or at reflux). Sat. aqueous NaOH (or LiOH) (2.5 mL) was added and the reaction was stirred at the chosen temperature between 30 minutes and 16 hours. The base was quenched with 10% aqueous HCl to pH 2 and the mixture was transferred to a separatory funnel with EtOAc (20 mL) and washed with H<sub>2</sub>O (20 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, evaporated to dryness *in vacuo* and dried overnight in high vacuum. This afforded Boc<sub>2</sub>-Phe-OH (**2.18**) as a pale, yellow solid (175 mg, 91%) (when NaOH was used at 5 °C for 7.5 hours). Characterization was in accordance with the previously synthesized compound. Enantiomeric excess was determined as described above (general method **C**) and it was found that in all cases basic hydrolysis led to complete racemization.

**General synthesis of phenylalanine-derived  $\gamma$ -amino- $\beta$ -keto esters (D)**

To a solution of Boc<sub>2</sub>-Phe-OH (**2.18**) (500 mg, 1.37 mmol, 1.0 equiv) in DCM (15 mL), Meldrum's acid (**2.1**) (296 mg, 2.05 mmol, 1.5 equiv) and DMAP (251 mg, 2.05 mmol, 1.5 equiv) were added. The mixture was cooled to 0 °C and EDC·HCl (393 mg, 2.05 mmol, 1.5 equiv) was added. Stirring was continued for 15 minutes and then at room temperature for 4 hours. The yellow reaction mixture was transferred to a separatory funnel with cold EtOAc (65 mL) and the organic phase was washed with cold brine (2 × 30 mL), cold 5% aqueous citric acid (2 × 30 mL) and finally with cold brine again (30 mL). The purification had to be done quickly and with cold solutions to avoid formation of the corresponding methyl ketone. The organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. This afforded the crude product as an oil, which was subsequently dissolved in a dry alcohol (15 mL) and the mixture was heated to reflux for 45 minutes. The desired  $\gamma$ -amino- $\beta$ -keto ester was isolated by evaporation to dryness *in vacuo* and thorough drying in vacuum.

**(4S)-Methyl 4,4-di(tert-butoxycarbonyl)amino-3-oxo-5-phenylpentanoate (Boc<sub>2</sub>-Phe-CH<sub>2</sub>COOMe) (2.21)**

Following the general synthesis of phenylalanine-derived  $\gamma$ -amino- $\beta$ -keto esters (D) afforded the product as a slightly yellow low-melting solid (541 mg, 94%) using dry MeOH for work-up of the intermediate. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.29–7.13 (m, 5H), 5.05 (dd, *J* = 9.8, 4.5, 1H), 3.73 (s, 3H), 3.57 (d, *J* = 18.0, 1H), 3.48 (d, *J* = 15.0, 1H), 3.46 (dd, *J* = 9.0, 6.0, 1H), 2.97 (dd, *J* = 15.0, 12.0, 1H), 1.39 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  198.8, 167.5, 151.3, 137.5, 129.5, 128.4, 126.6, 83.9, 66.0, 52.3, 45.2, 34.6, 27.7. HRMS (*m/z*) calcd. for C<sub>22</sub>H<sub>31</sub>NO<sub>7</sub>Na [M + Na]<sup>+</sup>: 444.1993, found: 444.1989. IR (neat)  $\nu$  2979, 2943, 1752 (strong), 1703 (strong), 1368, 1306, 1252, 1230, 1136, 1108, 873, 755, 697 cm<sup>-1</sup>.

**(4S)-Ethyl 4,4-di(tert-butoxycarbonyl)amino-3-oxo-5-phenylpentanoate (Boc<sub>2</sub>-Phe-CH<sub>2</sub>COOEt) (2.22)**

Following the general synthesis of phenylalanine-derived  $\gamma$ -amino- $\beta$ -keto esters (D) afforded the the product as an oil, which crystallized spontaneously upon storage at room temperature to a slightly yellow, low-melting solid (565 mg, 95%) using dry EtOH for work-up of the intermediate. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.29–7.13 (m, 5H), 5.06 (dd, *J* = 9.0, 3.0, 1H), 4.19 (q, *J* = 6.9, 2H), 3.56 (d, *J* = 15, 1H), 3.46 (d, *J* = 18, 1H), 3.46 (dd, *J* = 15.0, 3.0), 2.97 (dd, *J* = 14.3, 9.8, 1H), 1.39 (s, 19H), 1.27 (t, *J* = 7.2, 3H). <sup>13</sup>C (CDCl<sub>3</sub>)  $\delta$  198.9, 167.0, 151.2, 137.5, 129.5, 128.4, 126.5, 83.87, 65.9, 61.3, 45.3, 34.6, 27.7, 14.1. HRMS (*m/z*) calcd. for C<sub>23</sub>H<sub>33</sub>NO<sub>7</sub>Na [M + Na]<sup>+</sup>: 458.2149, found: 458.2160. Elem. anal. calcd. for C<sub>23</sub>H<sub>33</sub>NO<sub>7</sub>: C, 63.43; H, 7.64; N, 3.22, found: C, 63.06; H, 7.70; N, 3.38. IR (neat)  $\nu$  2979, 2943, 1752, 1704 (strong), 1688, 1367, 1306, 1250, 1136, 1108, 873, 697 cm<sup>-1</sup>.

**(4S)-Isopropyl 4,4-di(tert-butoxycarbonyl)amino-3-oxo-5-phenylpentanoate (Boc<sub>2</sub>-Phe-CH<sub>2</sub>COO<sup>i</sup>Pr) (2.23)**

Following the general synthesis of phenylalanine-derived  $\gamma$ -amino- $\beta$ -keto esters (**D**) afforded the product as a yellow viscous oil (604 mg, 98%) using dry <sup>i</sup>PrOH for work-up of the intermediate. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.29–7.13 (m, 5H), 5.09–5.03 (m, 2H), 3.52 (d,  $J$  = 15.0, 1H), 3.47 (dd,  $J$  = 14.3, 4.5, 1H), 3.44 (d,  $J$  = 15.0, 1H), 2.97 (dd,  $J$  = 14.1, 9.9, 1H), 1.39 (s, 18H), 1.27 (d,  $J$  = 3.0, 3H), 1.25 (d,  $J$  = 3.4, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  190.0, 166.6, 151.2, 137.5, 129.5, 128.4, 126.5, 83.9, 69.0, 66.0, 45.5, 34.6, 27.7, 21.7. HRMS ( $m/z$ ) calcd. for C<sub>24</sub>H<sub>35</sub>NO<sub>7</sub>Na [M + Na]<sup>+</sup>: 472.2306, found: 472.2306. Elem. anal. calcd. for C<sub>24</sub>H<sub>35</sub>NO<sub>7</sub>: C, 64.12; H, 7.85; N, 3.12, found: C, 63.96; H, 7.96; N, 3.42. IR (neat)  $\nu$  2981, 2936, 1793, 1721 (strong), 1697, 1455, 1366, 1307, 1240, 1133 (strong), 1104, 699 cm<sup>-1</sup>.

**(4S)-tert-Butyl 4,4-di(tert-butoxycarbonyl)amino-3-oxo-5-phenylpentanoate (Boc<sub>2</sub>-Phe-CH<sub>2</sub>COO<sup>t</sup>Bu) (2.24)**

Following the general synthesis of phenylalanine-derived  $\gamma$ -amino- $\beta$ -keto esters (**D**) afforded the the product as a yellow viscous oil (616 mg, 97%) using dry <sup>t</sup>BuOH for work-up of the intermediate. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.29–7.13 (m, 5H), 5.06 (dd,  $J$  = 9.8, 4.5, 1H), 3.50 (d,  $J$  = 15.0, 1H), 3.45 (dd,  $J$  = 15.0, 6.0, 1H), 3.36 (d,  $J$  = 15.0, 1H), 2.97 (dd,  $J$  = 15.0, 12.0, 1H), 1.46 (s, 9H), 1.39 (s, 19H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  199.4, 166.3, 151.2, 137.6, 129.5, 128.4, 126.5, 83.8, 82.0, 66.0, 46.3, 34.7, 27.97, 27.7. HRMS ( $m/z$ ) calcd. for C<sub>25</sub>H<sub>37</sub>NO<sub>7</sub>Na [M + Na]<sup>+</sup>: 486.2462, found: 486.2465. Elem. anal. calcd. for C<sub>25</sub>H<sub>37</sub>NO<sub>7</sub>: C, 64.77; H, 8.05; N, 3.02, found: C, 64.70; H, 8.17; N, 3.10. IR (neat)  $\nu$  2979, 2935, 1794, 1720 (strong), 1698, 1367, 1307, 1245, 1133 (strong), 963, 872 cm<sup>-1</sup>.

**Attempted synthesis of (4S)-benzyl 4,4-di(tert-butoxycarbonyl)amino-3-oxo-5-phenylpentanoate (Boc<sub>2</sub>-Phe-CH<sub>2</sub>COOBn) (2.25)**

Following the general synthesis of phenylalanine-derived  $\gamma$ -amino- $\beta$ -keto esters (**D**) afforded complete conversion (>95%) based on TLC analysis, R<sub>f</sub> = 0.85 (EtOAc), using dry BnOH for work-up of the intermediate. The excess BnOH could not be removed by evaporation *in vacuo* and the experiment was terminated.

**(2S)-Benzyl 2-(tert-butoxycarbonyl)amino-5-(3-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)guanidino)pentanoate (Boc-Arg(Pbf)-OBn) (2.26)**

Following the general synthesis of benzyl amino acids-derived esters (**B**) with Boc-Arg(Pbf)-OH and BnBr as starting materials, afforded the product as a white solid (386 mg, 88%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.40–7.26 (m, 5H), 6.28 (m, 2H), 5.34 (d,  $J$  = 7.2 Hz, 1H), 5.09 (s, 2H), 4.22 (m, 1H), 3.22–3.10 (m, 2H), 2.91 (s, 2H), 2.54 (s, 3H), 2.48 (s, 3H), 2.06 (s, 3H), 1.85–1.74 (m, 1H), 1.67–1.60 (m, 1H), 1.54–1.50 (m, 2H),



1.43 (s, 6H), 1.38 (s, 9H). HRMS ( $m/z$ ) calcd. for  $C_{31}H_{44}N_4O_5S$   $[M + H]^+$ : 617.3004, found: 617.2981. Mp 79–86 °C.

**(2S)-Allyl 5-(benzyloxycarbonyl)amino-2-(tert-butoxycarbonyl)amino-pentanoate (Boc-Orn(Cbz)-OAllyl) (2.27)**

Following the general synthesis of allyl amino acid-derived esters (**B**) with Boc-Orn(Cbz)-OH and allylbromide as starting materials, afforded the product as a pale, yellow oil (512 mg, 92%).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  7.36–7.31 (m, 5H), 5.96–5.84 (m, 1H), 5.29 (m, 2H), 5.09 (s, 2H), 4.91 (broad s, 1H), 4.63 (d,  $J = 5.7$  Hz, 2H), 4.33–4.29 (m, 1H), 3.22 (q,  $J = 6.2$  Hz, 2H), 1.96–1.83 (m, 1H), 1.72–1.51 (m, 3H), 1.44 (s, 9H). HRMS ( $m/z$ ) calcd. for  $C_{21}H_{30}N_2O_6Na$   $[M + Na]^+$ : 429.1996, found: 429.1988.

**(2S)-Allyl 6-(benzyloxycarbonyl)amino-2-(tert-butoxycarbonyl)amino-hexanoate (Boc-Lys(Cbz)-OAllyl) (2.28)**

Following the general synthesis of allyl amino acid-derived esters (**B**) with Boc-Lys(Cbz)-OH and allylbromide as starting materials, afforded the product as a pale, yellow oil (524 mg, 95%).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  7.36–7.29 (m, 5H), 5.97–5.84 (m, 1H), 5.36–5.23 (m, 2H), 5.09 (s, 3H), 4.91 (broad s, 1H), 4.63–4.61 (m, 2H), 4.30 (m, 1H), 3.19 (q,  $J = 6.4$  Hz, 2H), 1.88–1.71 (m, 1H), 1.69–1.62 (m, 1H), 1.56–1.48 (m, 2H), 1.43 (s, 9H), 1.42–1.32 (m, 2H). HRMS ( $m/z$ ) calcd. for  $C_{22}H_{32}N_2O_6Na$   $[M + Na]^+$ : 443.2152, found: 443.2155.

**Attempted regioselective  $N_\alpha$ -protection of Boc-Arg(Pbf)-OBn (2.26) by treatment with  $Boc_2O$  toward the synthesis of 2.29**

Following the general synthesis of  $N,N$ -Boc<sub>2</sub>-protected amino acids (**C**) (except the second step, the hydrogenolysis) with Boc-Arg(Pbf)-OBn (**2.26**) as the starting material. The reaction was very sluggish and full conversion could only be achieved by addition of several portions of 0.50 equiv  $Boc_2O$  over the course of 4 days. Unfortunately, this resulted in many undefined multiple Boc-protected products and extensively  $Boc_2O$  contamination. These findings were based on LC-MS analysis. MS ( $m/z$ ) calcd. for  $C_{36}H_{53}N_4O_9S$   $[M + H]^+$ : 717.35, found: 717.36.

**(2S)-Allyl 5,5-(benzyloxycarbonyl,tert-butoxycarbonyl)amino-2,2-di(tert-butoxycarbonyl)aminopropionic acid (Boc<sub>2</sub>-Orn(Boc,Cbz)-OAllyl) (2.30)**

Following the general synthesis of *N,N*-Boc<sub>2</sub>-protected amino acids (**C**) (except the second step, the hydrogenolysis) with Boc-Orn(Cbz)-OAllyl (**2.27**) as starting material with 2.20 equiv of Boc<sub>2</sub>O and 0.20 equiv of DMAP. The reaction was rather sluggish and many additional portions of 0.50 equiv Boc<sub>2</sub>O had to be added to secure full conversion based by LC-MS. The fully protected product was isolated as a brown, viscous oil (494 mg, 72%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.40–7.30 (m, 5H), 5.94–5.83 (m, 1H), 5.33–5.21 (m, 4H), 4.90 (m, 1H), 4.60 (d, *J* = 5.3 Hz, 2H), 3.69 (t, *J* = 7.0 Hz, 2H), 2.17–2.08 (m, 1H), 1.91–1.86 (m, 1H), 1.68–1.63 (m, 2H), 1.48 (s, 9H), 1.47 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 170.2, 153.8, 152.1, 151.9, 135.5, 131.8, 128.5, 128.1, 118.0, 83.0, 82.7, 68.3, 65.6, 57.8, 45.9, 27.9, 26.8, 25.8. HRMS (*m/z*) calcd. for C<sub>31</sub>H<sub>46</sub>N<sub>2</sub>O<sub>10</sub>Na [M + Na]<sup>+</sup>: 629.3044, found: 629.3029.

**(2S)-Allyl 6,6-(benzyloxycarbonyl, tert-butoxycarbonyl)amino-2,2-di(tert-butoxycarbonyl)aminohexanoic acid (Boc-Lys(Cbz)-OAllyl) (2.31)**

Following the general synthesis of *N,N*-Boc<sub>2</sub>-protected amino acids (**C**) (except the second step, the hydrogenolysis) with Boc-Lys(Cbz)-OAllyl (**2.28**) as starting material with 2.20 equiv Boc<sub>2</sub>O and 0.20 equiv of DMAP. The reaction was rather sluggish and many additional portions of 0.50 equiv Boc<sub>2</sub>O had to be added to secure full conversion based by LC-MS. The fully protected product was isolated as a brown, viscous oil (506 mg, 73%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.41–7.30 (m, 5H), 5.93–5.86 (m, 1H), 5.35–5.28 (m, 1H), 5.24–5.19 (m, 3H), 4.87–4.82 (m, 1H), 4.62–4.58 (m, 2H), 3.66–3.60 (m, 2H), 2.18–2.09 (m, 1H), 1.93–1.85 (m, 1H), 1.66–1.59 (m, 2H), 1.49 (s, 9H), 1.49–1.47 (m, 18H), 1.41–1.30 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 170.5, 153.9, 152.2, 152.0, 135.6, 131.9, 128.5, 128.2, 118.0, 83.0, 82.7, 68.2, 65.6, 58.1, 46.4, 29.4, 28.7, 28.0, 23.6. HRMS (*m/z*) calcd. for C<sub>32</sub>H<sub>48</sub>N<sub>2</sub>O<sub>10</sub>Na [M + Na]<sup>+</sup>: 643.3201, found: 643.3199.

**(5S)-1-(tert-Butyloxycarbonyl)-4-hydroxy-5-(3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino)-propyl)-1,5-dihydro-pyrrol-2-one (Boc-pyArg(Pbf)-OH) (2.32)**

Following the general synthesis of tetramic acids (**A**) using Boc-Arg(Pbf)-OH as amino acid starting material, afforded the product as a pale, yellow solid (6.80 g, 99%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 12.30 (broad s, 1H), 6.73 (broad s, 1H), 6.40 (broad s, 2H), 4.87 (s, 1H), 4.39 (m, 1H), 3.03 (q, *J* = 6.6 Hz, 2H), 2.96 (s, 2H), 2.47 (s, 3H), 2.42 (s, 3H), 2.01 (s, 3H), 1.95–1.89 (m, 1H), 1.81–1.73 (m, 1H), 1.42 (s, 9H), 1.41 (s, 6H), 1.25–1.15 (m, 2H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 176.6, 169.2, 157.5, 156.0, 148.7, 137.3, 134.1, 131.4, 124.3, 116.3, 94.1, 86.3, 80.9, 58.9, 42.5, 28.3, 27.7, 26.3, 18.9, 17.6, 12.3. HRMS (*m/z*) calcd. for C<sub>26</sub>H<sub>39</sub>N<sub>4</sub>O<sub>7</sub>S [M + H]<sup>+</sup>: 551.2534, found: 551.2537. HPLC purity (TFA): >78.8%. Mp 142–148 °C. IR (neat): ν 3441, 3332, 2973, 2930, 1755, 1617, 1548 (strong), 1367, 1299, 1240, 1152, 1083 cm<sup>-1</sup>.



**(5S)-5-(4-tert-Butoxybenzyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-pyTyr(<sup>t</sup>Bu)-OH) (2.33)**

Following the general synthesis of tetramic acids (**A**) using Boc-Tyr(<sup>t</sup>Bu)-OH as amino acid starting material, afforded the product in quantitative yield as a pale, yellow foam (4.81 g, 106%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 12.34 (broad s, 1H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.82 (d, *J* = 8.0 Hz, 2H), 4.69 (s, 1H), 4.57 (dd, *J* = 5.2, 2.4 Hz, 1H), 3.32 (dd, *J* = 13.9, 5.3 Hz, 1H), 3.03 (dd, *J* = 13.9, 2.3 Hz, 1H), 1.50 (s, 9H), 1.24 (s, 9H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 175.5, 168.7, 153.8, 149.0, 130.1, 129.1, 123.1, 94.9, 80.9, 77.6, 59.8, 33.7, 28.5, 27.9. HRMS (*m/z*) calcd. for C<sub>20</sub>H<sub>27</sub>NO<sub>5</sub>Na [M + Na]<sup>+</sup>: 384.1781, found: 384.1793. HPLC purity (TFA): 82%. Mp 73–76 °C. IR (neat): ν 2976, 2932, 1754 (strong), 1710, 1607, 1505, 1363, 1234, 1148 (strong), 1074 cm<sup>-1</sup>.

**(5S)-5-(4-(Benzyloxycarbonyl)butyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-pyLys(Cbz)-OH) (2.34)**

Following the general synthesis of tetramic acids (**A**) using Boc-Lys(Cbz)-OH as amino acid starting material, afforded the product as a pale, yellow foam (800 mg, 94%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 12.24 (broad s, 1H), 7.38–7.28 (m, 5H), 5.00 (s, 2H), 4.89 (s, 1H), 4.39 (dd, *J* = 5.1, 2.5 Hz, 1H), 2.96 (q, *J* = 6.5 Hz, 2H), 2.03–1.95 (m, 1H), 1.81–1.75 (m, 1H), 1.44 (s, 9H), 1.41–1.35 (m, 2H), 1.17–1.03 (m, 2H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) 176.7, 169.3, 156.1, 148.8, 137.3, 128.31, 127.7, 94.2, 80.8, 65.1, 59.2, 40.3, 29.4, 28.5, 27.8, 19.1. HRMS (*m/z*) calcd. for C<sub>42</sub>H<sub>56</sub>N<sub>4</sub>O<sub>12</sub>Na [2M + Na]<sup>+</sup>: 831.3786, found: 831.3790. HPLC purity (TFA): 83%. Mp 43–46 °C. IR (neat): ν 3324, 2974, 2933, 1756, 1702 (strong), 1613, 1530, 1365, 1299, 1246, 1152 (strong), 1076 cm<sup>-1</sup>.

**(5S)-5-((1S)-1-tert-Butoxyethyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-pyThr(<sup>t</sup>Bu)-OH) (2.35)**

Following the general synthesis of tetramic acids (**A**) using Boc-Thr(<sup>t</sup>Bu)-OH as amino acid starting material, afforded the product in quantitative yield as a viscous, yellow oil (3.73 g, 100%), which solidified upon storage to a low-melting solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.71 (s, 1H), 4.89 (s, 1H), 4.31 (d, *J* = 3.5 Hz, 1H), 4.19 4.13 (m, 1H), 1.45 (s, 9H), 1.15 (s, 9H), 0.95 (d, *J* = 6.3 Hz, 3H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 176.3, 169.0, 149.3, 94.8, 81.1, 73.7, 65.6, 63.3, 27.9, 27.8, 17.5. HRMS (*m/z*) calcd. for C<sub>15</sub>H<sub>25</sub>NO<sub>5</sub>Na [M + Na]<sup>+</sup>: 322.1625, found: 322.1628. HPLC purity (TFA): >85.8%. IR (neat): ν 2977, 2933, 1704 (strong), 1677, 1586, 1306 (strong), 1285, 1150, 1093 cm<sup>-1</sup>.

**(5S)-5-(2-tert-Butoxy-2-oxoethyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-pyAsp(<sup>t</sup>Bu)-OH) (2.36)**

Following the general synthesis of tetramic acids (**A**) using Boc-Asp(<sup>t</sup>Bu)-OH as amino acid starting material, afforded the product as a pale, yellow solid (3.68 g, 94%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  12.39 (s, 1H), 4.85 (s, 1H), 4.56 (dd, *J* = 6.3, 2.8 Hz, 1H), 2.81 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.73 (dd, *J* = 16.0, 4 Hz, 1H), 1.45 (s, 9H), 1.33 (s, 9H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  175.8, 168.8, 167.9, 148.6, 93.9, 80.9, 80.1, 56.7, 35.9, 27.7, 27.5. HRMS (*m/z*) calcd. for C<sub>30</sub>H<sub>46</sub>N<sub>2</sub>O<sub>12</sub>Na [2M + Na]<sup>+</sup>: 649.2942, found: 649.2932. HPLC purity (TFA): >75.2%. Mp 73–76 °C. IR (neat):  $\nu$  2978, 2943, 1738, 1716 (strong), 1368, 1317, 1245, 1149, 848 cm<sup>-1</sup>.

**(5S)-1-(tert-Butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-pyGly-OH) (2.37)**<sup>325</sup>

Following the general synthesis of tetramic acids (**A**) using Boc-Gly-OH as amino acid starting material, afforded the product as a pale, yellow solid (2.47 g, 99%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  12.17 (broad s, 1H), 4.87 (s, 1H), 4.13 (s, 2H), 1.43 (s, 9H). HRMS (*m/z*) calcd. for C<sub>9</sub>H<sub>13</sub>NO<sub>4</sub>Na [M + Na]<sup>+</sup>: 222.0737, found: 222.0745. HPLC purity (TFA): >97.3%. Mp 123–127 °C (lit.:<sup>325</sup> N/A).

**(5S)-5-(4-(Benzyloxycarbonyl)propyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (Boc-pyOrn(Cbz)-OH) (2.38)**

Following the general synthesis of tetramic acids (**A**) using Boc-Orn(Cbz)-OH as starting material, afforded the product as a white solid (804 mg, 94%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.37–7.30 (m, 5H), 5.17–5.03 (m, 3H), 4.87 (broad s, 1H), 4.49–4.39 (m, 1H), 3.24–3.07 (m, 3H), 2.17–1.88 (m, 2H), 1.72–1.60 (m, 1H), 1.54, 1.51 (2  $\times$  s, 9H), 1.46–1.41 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  204.0, 172.6, 167.4, 156.9, 156.4, 149.3, 149.0, 136.4, 136.1, 128.6, 128.2, 128.1, 84.5, 83.0, 67.0, 66.7, 59.9, 42.9, 40.3, 28.1, 28.0, 26.3, 24.8, 22.9. HRMS (*m/z*) calcd. for C<sub>20</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub>Na [M + Na]<sup>+</sup>: 413.1683, found: 413.1689. Mp 48–54 °C.

**General synthesis of  $\alpha$ -aryl tetramic acids (**E**)**

A pressure resistant glass vial was charged with a magnetic stir bar, dry THF (3.0 mL), tetramic acid (1.00 mmol, 1.00 equiv), <sup>t</sup>Bu-XPhos (**2.44**) (17 mg, 0.04 mmol, 0.04 equiv), K<sub>2</sub>CO<sub>3</sub> (318 mg, 2.30 mmol, 2.30 equiv) and an aryl chloride coupling partner (1.00 mmol, 1.00 equiv). N<sub>2</sub> was bubbled through the reaction mixture and Pd(OAc)<sub>2</sub> (4 mg, 0.02 mmol, 0.02 equiv) was added, the vial was filled with N<sub>2</sub>, sealed with a screw cap and placed in an aluminum heating block. The mixture was stirred vigorously at 80 °C for 1 hour (or 16 hours, c.f. Tables 2.2.12 and 2.2.13). After cooling to ambient temperature, the crude mixture was transferred to a separatory funnel with 10% aqueous KHSO<sub>4</sub> (10 mL) and extracted with EtOAc (30 mL + 20 mL). The combined organic phases were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*.

The yellow crude product was purified by flash chromatography (5→10 vol% MeOH in EtOAc, in some cases up to 20 vol% MeOH) affording the pure product typically as a keto/enol tautomer mixture. The product was subsequently suspended in EtOAc (50 mL). 10% aqueous KHSO<sub>4</sub> (50 mL) was added and the biphasic system was stirred vigorously at room temperature until complete dissolution of the compound. The mixture was transferred to a separatory funnel and the organic layer was separated. The aqueous layer was extracted with EtOAc (20 mL) and the combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, evaporated to dryness *in vacuo* and dried overnight in high vacuum which afforded the pure product mostly as the enol-tautomer.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.40)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 4-chloroanisole (**2.53**) as coupling partners, afforded the product as a pale, brown solid (313 mg, 79%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  12.35 (broad s, 1H), 7.22–7.12 (m, 6H), 6.99 (d, *J* = 6.8 Hz, 2H), 6.76 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.73 (dd, *J* = 4.6, 2.5 Hz, 1H), 3.70 (s, 3H), 3.45 (dd, *J* = 14.0, 4.9 Hz, 1H), 3.27 (dd, *J* = 13.9, 2.3 Hz, 1H), 1.54 (s, 9H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  170.4, 167.4, 158.7, 149.0, 134.1, 131.8, 129.5, 128.7, 127.9, 126.8, 120.0, 113.2, 111.7, 105.1, 81.3, 57.9, 54.8, 34.6, 27.9. HRMS (*m/z*) calcd for C<sub>23</sub>H<sub>25</sub>NO<sub>5</sub>: 813.3358, found: 813.3367. Elem. anal. calcd. for C<sub>23</sub>H<sub>25</sub>NO<sub>5</sub>: C, 69.86; H, 6.37; N, 3.54, found: C, 69.84; H, 6.52; N, 3.49. HPLC purity (TFA): >98.1%. Mp 142–145 °C. IR (neat):  $\nu$  2975, 2930, 1750 (strong), 1363, 1284, 1251, 1147, 1095, 833, 699 cm<sup>-1</sup>. Chiral HPLC: 4.57 minutes (minor) and 5.78 minutes (major) gave an enantiomeric excess of 97%.

**Microwave assisted synthesis of (5S)-5-benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.40)**

A microwave vial was charged with Boc-*py*Phe-OH (**2.4**) (289 mg, 1.00 mmol, 1.00 equiv), *t*Bu-XPhos (**2.44**) (17 mg, 0.04 mmol, 0.04 equiv), Pd(OAc)<sub>2</sub> (4 mg, 0.02 mmol, 0.02 equiv) and K<sub>2</sub>CO<sub>3</sub> (318 mg, 2.30 mmol, 2.30 equiv). Dry THF (3.0 mL) was added and used to carefully rinse the inside of the vial (for safety reasons no solid may be stuck on the glass!). 4-Chloroanisole (**2.53**) (143 mg, 0.08 mL, 1.00 equiv) was added and N<sub>2</sub> was bubbled into the vial to secure an inert reaction atmosphere. The vial was capped, sealed and heated to 110 °C in a microwave synthesizer for 5 minutes. After cooling to ambient temperature, the crude product was neutralized with 10% aqueous KHSO<sub>4</sub> (10 mL) and EtOAc (30 + 20 mL) was added. The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. The crude product was analyzed by <sup>1</sup>H NMR in DMSO-*d*<sub>6</sub> which showed full conversion (>95%) and the spectrum was identical with that of the product obtained by conventional heating (80 °C, 1 hour).

**4-Methoxyphenyl tosylate (2.55)**<sup>326</sup>

To a solution of 4-methoxyphenol (1.24 g, 10.00 mmol, 1.00 equiv) in pyridine (10 mL), TsCl (2.10 g, 11.00 mmol, 1.10 equiv) was added portionwise at room temperature and the mixture was stirred at 45 °C overnight. After cooling to ambient temperature, H<sub>2</sub>O (25 mL) was added and stirred at room temperature for 3 hours. This mixture was diluted with toluene (200 mL) and washed with H<sub>2</sub>O (150 mL), 10% HCl (3 × 150 mL), sat. aqueous NaHCO<sub>3</sub> (2 × 150 mL) and finally with brine (2 × 150 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated *in vacuo* to an oil, which crystallized upon drying in high vacuum. The tosylate **2.55** was obtained as a white solid (2.62 g, 94%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.70 (d, *J* = 7.3 Hz, 2H), 7.45 (d, *J* = 7.6 Hz), 6.92–6.87 (m, 4H), 3.70 (s, 3H), 2.40 (s, 3H). HRMS (*m/z*) calcd. for C<sub>14</sub>H<sub>14</sub>O<sub>4</sub>SNa [M + Na]<sup>+</sup>: 301.0510, found: 301.0410. Mp 66–71 °C (lit.:<sup>326</sup> 69–70 °C).

**4-Methoxyphenyl triflate (2.56)**<sup>327</sup>

To a cooled (0 °C) mixture of toluene (20 mL) and 30% aqueous K<sub>3</sub>PO<sub>4</sub> (20 mL) was added 4-methoxyphenol (1.24 g, 10.00 mmol, 1.00 equiv). Tf<sub>2</sub>O (1.34 mL, 2.29 g, 12.00 mmol, 1.10 equiv) was added dropwise under vigorously stirring at a rate to maintain the reaction temperature below 10 °C. The reaction was allowed to warm ambient temperature and stirred for 30 minutes. The organic phase was separated and then washed with H<sub>2</sub>O (20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. The product **2.56**, which was a yellow liquid, was dried in high vacuum overnight (1.80 g, 70%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.41 (d, *J* = 9.09 Hz, 2H), 7.08 (d, *J* = 9.09 Hz, 2H), 3.79 (s, 3H). Contained 10% of the 4-methoxyphenol starting material: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 6.74 (m, 2H), 6.68 (m, 2H), 3.65 (s, 3H).

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-phenyl-1,5-dihydro-pyrrol-2-one (2.57)**

Following the general synthesis of α-aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and chlorobenzene as coupling partners, afforded the product as a pale, brown solid (273 mg, 75%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 12.30 (broad s, 1H), 7.52 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.22–7.15 (m, 4H), 7.01–6.98 (m, 2H), 4.73 (dd, *J* = 4.8, 2.5 Hz, 1H), 3.45 (dd, *J* = 13.9, 4.8 Hz, 1H), 3.27 (dd, *J* = 14.0, 2.4 Hz, 1H), 1.54 (s, 9H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 170.2, 167.4, 149.0, 134.2, 130.5, 129.5, 127.9, 127.7, 127.5, 126.8, 126.3, 105.4, 81.2, 57.9, 34.6, 27.9. HRMS (*m/z*) calcd. for C<sub>44</sub>H<sub>46</sub>N<sub>2</sub>O<sub>8</sub>Na [2M + Na]<sup>+</sup>: 753.3146, found: 753.3153. Elem. anal. calcd. for C<sub>22</sub>H<sub>23</sub>NO<sub>4</sub>: C, 72.31; H, 6.34; N, 3.83, found: C, 71.97; H, 6.60; N, 3.96. Mp 86–88 °C. IR (neat): ν 3082, 3061, 2977, 2928, 1753, 1702, 1661, 1645 (strong), 1397, 1359, 1298, 1149, 694 cm<sup>-1</sup>.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(3-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.58)**

Following the general synthesis of α-aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**)

and 3-chloroanisole as coupling partners, afforded the product as a pale, brown solid (310 mg, 78%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  12.35 (broad s, 1H), 7.22–7.12 (m, 6H), 6.99 (d,  $J$  = 6.8 Hz, 2H), 6.76 (dd,  $J$  = 8.0, 1.4 Hz, 1H), 4.72 (dd,  $J$  = 4.4, 2.4 Hz, 1H), 3.70 (s, 3H), 3.45 (dd,  $J$  = 13.9, 4.8 Hz, 1H), 3.27 (dd,  $J$  = 13.9, 2.0 Hz, 1H), 1.54 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  170.4, 167.4, 158.7, 149.0, 134.1, 131.8, 129.5, 128.7, 127.9, 126.8, 120.0, 113.2, 111.7, 105.1, 81.3, 57.9, 54.8, 34.6, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{46}\text{H}_{50}\text{N}_2\text{O}_{10}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 813.3358, found: 813.3377. Elem. anal. calcd. for  $\text{C}_{23}\text{H}_{25}\text{NO}_5$ : C, 69.86; H, 6.37; N, 3.54, found: C, 69.65; H, 6.60; N, 3.46. Mp 136–138 °C. IR (neat):  $\nu$  3196, 2974, 2929, 1736 (strong), 1680, 1653, 1407, 1340, 1308, 1147, 1108, 755, 700  $\text{cm}^{-1}$ .

**Attempted synthesis of (5S)-5-benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(2-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.59) with 2-chloro- anisole as coupling partner**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 2-chloroanisole as coupling partners (16 hours), did not afford any significant product based on LC-MS and  $^1\text{H}$  NMR.

**Attempted synthesis of (5S)-5-benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(2-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.59) with 2-bromo- anisole as coupling partner**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 2-bromoanisole as coupling partners (16 hours), did not afford any significant product formation based on LC-MS and  $^1\text{H}$  NMR.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-hydroxyphenyl)-1,5-dihydro-pyrrol-2-one (2.60)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 4-chlorophenol as coupling partners and 16 hours reaction time, afforded the product as a pale, brown solid (273 mg, 72%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  11.95 (broad s, 1H), 9.34 (broad s, 1H), 7.36 (d, 2H,  $J$  = 8.6 Hz), 7.22–7.15 (m, 3H), 6.99 (d,  $J$  = 6.8 Hz, 2H), 6.69 (d,  $J$  = 8.6 Hz, 2H), 4.68 (dd,  $J$  = 4.7, 2.0 Hz, 1H), 3.43 (dd,  $J$  = 13.8, 4.7 Hz, 1H), 3.26 (dd,  $J$  = 13.5, 2.0 Hz, 1H), 1.53 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  168.3, 167.8, 155.9, 149.1, 134.3, 129.6, 128.8, 127.9, 126.8, 126.8, 121.2, 114.6, 105.6, 81.2, 57.8, 34.6, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{44}\text{H}_{46}\text{N}_2\text{O}_{10}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 785.3044, found: 785.3046. Elem. anal. calcd. for  $\text{C}_{22}\text{H}_{23}\text{NO}_5$ : C, 69.28; H, 6.08; N, 3.67, found: C, 69.49; H, 6.31; N, 3.52. Mp 136–139 °C. IR (neat):  $\nu$  3370 (broad), 2978, 2932, 1726 (strong), 1516, 1396, 1358, 1147, 812, 700  $\text{cm}^{-1}$ .

**(5S)-3-(4-Aminophenyl)-5-benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.61)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 4-chloroaniline as coupling partners and 16 hours reaction time, afforded the desired product (conv. >95%) based on  $^1\text{H}$  NMR, which was not further purified.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.37 (d,  $J$  = 8.6 Hz, 2H), 7.21–7.14 (m, 3H), 6.97 (d,  $J$  = 7.8 Hz, 2H), 6.68 (d,  $J$  = 8.3 Hz, 2H), 6.67 (s, 1H), 4.66 (dd,  $J$  = 4.8, 2.5 Hz, 1H), 3.41 (dd,  $J$  = 13.9, 5.1 Hz, 1H), 3.24 (dd,  $J$  = 14.0, 1.9 Hz, 1H), 1.52 (s, 9H). HRMS ( $m/z$ ) calcd. for  $\text{C}_{44}\text{H}_{48}\text{N}_4\text{O}_8\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 783.3364, found: 783.3367.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-3-(4-tert butyl phenylcarbamate)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.62)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and *N*-tert-butyl 4-chlorophenylcarbamate as coupling partners, afforded the product as a brown solid (360 mg, 75%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  12.09 (broad s, 1H), 9.29 (s, 1H), 7.45 (d,  $J$  = 8.5, 2H), 7.36 (d,  $J$  = 8.6, 2H), 7.23–7.13 (m, 3H), 6.99 (d,  $J$  = 6.82, 1H), 4.70 (dd,  $J$  = 4.7, 2.4 Hz, 1H), 3.44 (dd,  $J$  = 13.9, 4.8 Hz, 1H), 3.26 (dd,  $J$  = 13.9, 2.0 Hz, 1H), 1.53 (s, 9H), 1.47 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  169.1, 167.5, 152.7, 149.1, 137.8, 134.2, 129.5, 127.7, 127.7, 126.7, 124.4, 117.5, 105.2, 81.2, 78.9, 57.9, 34.6, 28.1, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{54}\text{H}_{64}\text{N}_4\text{O}_{12}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 983.4412, found: 983.4403. HPLC purity (TFA): >94.6%. Mp 140–143 °C. IR (neat):  $\nu$  3359, 2976, 2931, 1750, 1732, 1590, 1522, 1392, 1367, 1309, 1228, 1228, 1149 (strong)  $\text{cm}^{-1}$ .

**Attempted synthesis of (5S)-5-benzyl-1-(tert-butyloxycarbonyl)-3-(4-cyanophenyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.63)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 2-(4-chlorophenyl)ethaneamine as coupling partners, did not afford any significant product formation based on LC-MS and  $^1\text{H}$  NMR.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-3-(4-cyanophenyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.64)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 4-chlorobenzonitrile as coupling partners, afforded the product as a pale, brown solid (288 mg, 74%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.88 (d,  $J$  = 8.6 Hz, 2H), 7.72 (d,  $J$  = 8.6 Hz, 2H), 7.20–7.13 (m, 3H), 6.96 (d,  $J$  = 6.8 Hz, 2H), 4.75 (dd,  $J$  = 4.8, 2.5 Hz, 1H), 3.44 (dd,  $J$  = 14.0, 4.9 Hz, 1H), 3.28 (dd,  $J$  = 14.0, 2.4 Hz, 1H), 1.53 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  173.6, 167.0, 148.9, 136.0, 134.1, 131.7, 129.4, 127.9, 127.1, 126.8, 119.1, 108.0, 103.0, 81.4, 58.3, 34.7, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{46}\text{H}_{44}\text{N}_4\text{O}_8\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 803.3052, found: 803.3025. HPLC purity (TFA): >94.4%. Mp 225–230 °C (decomp.). IR (neat):  $\nu$  2974, 2928, 2225, 1748 (strong), 1644, 1603, 1352 (strong), 1302 (strong), 1149, 699, 550  $\text{cm}^{-1}$ .

**Attempted synthesis of (5S)-5-benzyl-1-(tert-butyloxycarbonyl)-3-(2-cyano-phenyl)-**



**4-hydroxy-1,5-dihydro-pyrrol-2-one (2.65)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 2-chlorobenzonitrile as coupling partners (16 hours), did not afford any significant product formation based on LC-MS and  $^1\text{H}$  NMR.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-nitrophenyl)-1,5-dihydro-pyrrol-2-one (2.66)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 4-nitrobenzene as coupling partners and 16 hours reaction time, afforded the product as a pale, brown solid (244 mg, 60%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.15 (d,  $J = 9.1$  Hz, 2H), 8.01 (d,  $J = 9.1$  Hz, 2H), 7.20–7.11 (m, 3H), 6.97 (d,  $J = 6.6$  Hz, 2H), 4.78 (dd,  $J = 4.8, 2.5$  Hz, 1H), 3.46 (dd,  $J = 14.0, 4.9$  Hz, 1H), 3.29 (dd,  $J = 14.0, 2.4$  Hz, 1H), 1.54 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  174.6, 167.0, 148.9, 144.6, 138.3, 134.0, 129.4, 127.9, 127.0, 126.8, 123.1, 102.6, 81.4, 58.4, 34.7, 27.8. HRMS ( $m/z$ ) calcd. for  $\text{C}_{44}\text{H}_{44}\text{N}_4\text{O}_{12}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 843.2848, found: 843.2863. Elem. anal. calcd. for  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_6$ : C, 64.38; H, 5.40; N, 6.83, found: C, 64.61; H, 5.65; N, 6.52. Mp approx. 250  $^\circ\text{C}$  (decomp.). IR (neat):  $\nu$  2973, 2931, 2225, 1761, 1714, 1600, 1425, 1340 (strong), 1146, 850, 702  $\text{cm}^{-1}$ .

**(5S)-3-(4-Acetylphenyl)-5-benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.67)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 1-(4-chlorophenyl)ethanone as coupling partners, afforded the product as a pale, brown solid (302 mg, 74%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.87 (d,  $J = 8.0$  Hz, 2H), 7.79 (d,  $J = 8.0$  Hz, 2H), 7.21–7.14 (m, 3H), 6.97 (d,  $J = 6.6$  Hz, 2H), 4.77 (dd,  $J = 4.8, 2.5$  Hz, 1H), 3.45 (dd,  $J = 13.9, 5.1$  Hz, 1H), 3.28 (dd,  $J = 14.0, 2.4$  Hz, 1H), 2.53 (s, 3H), 1.54 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  197.3, 172.5, 167.1, 149.0, 135.7, 134.3, 134.1, 129.5, 127.9, 127.8, 126.8, 104.0, 81.4, 58.2, 34.7, 27.9, 26.6. HRMS ( $m/z$ ) calcd. for  $\text{C}_{48}\text{H}_{50}\text{N}_2\text{O}_{10}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 837.3358, found: 837.3345. Elem. anal. calcd. for  $\text{C}_{24}\text{H}_{25}\text{NO}_5$ : C, 70.74; H, 6.18; N, 3.44, found: C, 70.89; H, 6.43; N, 3.46. Mp 227–230  $^\circ\text{C}$  (decomp.). IR (neat):  $\nu$  2973, 2926, 1763, 1713, 1680 (strong), 1603 (strong), 1357, 1264 (strong), 1143  $\text{cm}^{-1}$ .

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-3-(4-carboxyphenyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.68)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 4-chlorobenzoic acid as coupling partner and 16 hours reaction time, afforded the desired product (conv. >95%) based on  $^1\text{H}$  NMR, which was not further purified.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  12.79 (broad s, 1H), 7.86 (d,  $J$  = 8.0 Hz, 2H), 7.75 (d,  $J$  = 8.0 Hz, 2H), 7.21–7.14 (m, 3H), 6.98 (d,  $J$  = 6.8 Hz, 2H), 4.77 (dd,  $J$  = 4.6, 2.5 Hz, 1H), 3.46 (dd,  $J$  = 14.0, 4.9 Hz, 1H), 3.29 (dd,  $J$  = 13.9, 2.0 Hz, 1H), 1.54 (s, 9H). HRMS ( $m/z$ ) calcd. for  $\text{C}_{46}\text{H}_{46}\text{N}_2\text{O}_{12}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 841.2942, found: 841.2942.

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-(methoxycarbonyl)phenyl)-1,5-dihydro-pyrrol-2-one (2.69)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and methyl 4-chlorobenzoate as coupling partner, afforded the product as a pale, brown solid (317 mg, 75%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.88 (d,  $J$  = 8.0 Hz, 2H), 7.80 (d,  $J$  = 8.0 Hz, 2H), 7.23–7.14 (m, 3H), 6.99 (d,  $J$  = 7.8 Hz, 2H), 4.78 (dd,  $J$  = 4.8, 2.5 Hz, 1H), 3.82 (s, 3H), 3.47 (dd,  $J$  = 13.9, 5.1 Hz, 1H), 3.30 (dd,  $J$  = 14.0, 2.4 Hz, 1H), 1.54 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  172.6, 167.1, 166.1, 149.0, 135.9, 134.1, 131.0, 129.5, 128.7, 127.9, 126.9, 103.8, 81.4, 58.2, 52.0, 34.6, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{48}\text{H}_{50}\text{N}_2\text{O}_{12}\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 869.3256, found: 869.3235. Elem. anal. calcd. for  $\text{C}_{24}\text{H}_{25}\text{NO}_6$ : C, 68.07; H, 5.95; N, 3.31, found: C, 68.01; H, 5.93; N, 3.51. Mp 210–212 °C (decomp.). IR (neat):  $\nu$  2951, 2930, 1751, 1715 (strong), 1605, 1355, 1274 (strong), 1149, 1079, 699  $\text{cm}^{-1}$ .

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-3-(4-chlorophenyl)-4-hydroxy-1,5-dihydro-pyrrol-2-one (2.70)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Phe-OH (**2.4**) and 1-bromo-4-chlorobenzene as coupling partner, afforded the product as a pale, brown solid (307 mg, 77%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.64 (d,  $J$  = 8.8 Hz, 2H), 7.35 (d,  $J$  = 8.6 Hz, 2H), 7.22–7.13 (m, 3H), 6.98 (d,  $J$  = 6.6 Hz, 2H), 4.75 (dd,  $J$  = 4.9, 2.7 Hz, 1H), 3.46 (dd,  $J$  = 14.0, 4.9 Hz, 1H), 3.28 (dd,  $J$  = 14.2, 2.5 Hz, 1H), 1.53 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  170.8, 167.2, 149.0, 134.1, 130.7, 129.5 (two peaks), 128.9, 127.9, 127.8, 126.8, 104.0, 81.4, 58.0, 34.6, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{44}\text{H}_{44}\text{Cl}_2\text{N}_2\text{O}_8\text{Na}$  [ $2\text{M} + \text{Na}$ ] $^+$ : 821.2366, found: 821.2360. MS ( $m/z$ ) for [ $2\text{M} + \text{Na}$ ] $^+$ : 821.20 (26%), 822 (9%), 823.21 (20%), 824 (4%). HPLC purity (TFA): >97.0%. Mp 240–244 °C (decomp.). IR (neat):  $\nu$  3027, 2926, 2680, 1750 (strong), 1611, 1587, 1390, 1361, 1273, 1150, 1090, 825, 677  $\text{cm}^{-1}$ .



**(5S)-1-(tert-Butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-5-(3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino)-propyl)-1,5-dihydro-pyrrol-2-one (2.71)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Arg(Pbf)-OH (**2.32**) and 4-chloroanisole (**2.53**) as coupling partners and 16 hours reaction time, afforded the product as a pale, brown solid (437 mg, 67%). Purification required approx. 15 vol% MeOH in EtOAc to eluate the product completely.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  11.96 (broad s, 1H), 7.75 (d,  $J$  = 9.1 Hz, 2H), 6.92 (d,  $J$  = 8.8 Hz, 2H), 6.71 (s, broad), 6.36 (s, broad), 4.53 (t,  $J$  = 3.5 Hz, 1H), 3.75 (s, 3H), 3.07–2.98 (m, 2H), 2.92 (s, 3H), 2.46 (s, 3H), 2.40 (s, 3H), 2.13–2.04 (m, 1H), 1.99 (s, 4H), 1.95–1.88 (m, 1H), 1.46 (s, 9H), 1.40 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  170.0, 168.2, 157.7, 157.5, 155.9, 148.7, 137.3, 134.0, 131.5, 128.6, 124.3, 123.0, 116.3, 113.3, 104.1, 86.3, 81.1, 59.7, 57.0, 55.0, 42.4, 28.2, 27.7, 26.2, 20.7, 18.9, 17.5, 14.0, 12.2. HRMS ( $m/z$ ) calcd. for  $\text{C}_{33}\text{H}_{45}\text{N}_4\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$ : 657.2953, found: 657.2961. HPLC purity (TFA): >88.6%. Mp 172–174 °C. IR (neat):  $\nu$  3445, 3332, 2969, 2928, 1749, 1610, 1550 (strong), 1514, 1350, 1292, 1246, 1153, 1089 (strong), 834, 781, 660, 566  $\text{cm}^{-1}$ .

**(5S)-5-(4-tert-Butoxybenzyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.72)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Tyr( $t$ Bu)-OH (**2.33**) and 4-chloroanisole (**2.53**) as coupling partners, afforded the product as a pale, brown solid (324 mg, 69%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  12.02 (s, 1H), 7.42 (d,  $J$  = 9.1 Hz, 2H), 6.88 (d,  $J$  = 8.6 Hz, 2H), 6.83 (d,  $J$  = 9.1 Hz, 2H), 6.77 (d,  $J$  = 8.6 Hz), 4.66 (dd,  $J$  = 4.6, 2.8 Hz, 1H), 3.71 (s, 3H), 3.39 (dd,  $J$  = 13.9, 4.6 Hz, 1H), 3.19 (dd,  $J$  = 13.9, 2.5 Hz, 1H), 1.53 (s, 9H), 1.16 (s, 9H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  168.8, 167.6, 157.7, 153.7, 149.0, 130.0, 128.9, 128.7, 123.2, 122.8, 113.1, 105.4, 81.1, 77.7, 57.9, 55.0, 34.1, 28.4, 27.9. HRMS ( $m/z$ ) calcd. for  $\text{C}_{54}\text{H}_{66}\text{N}_2\text{O}_{12}\text{Na}$   $[2\text{M} + \text{Na}]^+$ : 957.4508, found: 957.4516. HPLC purity (TFA): >91.7%. Mp 147–153 °C. IR (neat):  $\nu$  2975, 2932, 1748, 1643, 1607, 1514, 1392, 1363, 1290, 1247, 1150 (strong), 1095, 894, 830  $\text{cm}^{-1}$ .

**(5S)-5-(4-(Benzyloxycarbonyl)butyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.73)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Lys(Cbz)-OH (**2.34**) and 4-chloroanisole (**2.53**) as coupling partners, afforded the product as a brown solid (353 mg, 69%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  11.90 (broad s, 1H), 7.76 (d,  $J$  = 9.1 Hz, 2H), 7.37–7.29 (m, 5H), 6.92 (d,  $J$  = 8.8 Hz, 2H), 4.97 (s, 2H), 4.52 (dd,  $J$  = 4.2, 3.2 Hz, 1H), 3.75 (s, 3H), 2.94 (q,  $J$  = 6.6 Hz, 2H), 2.16–2.07 (m, 1H), 1.98–1.90 (m, 1H), 1.47 (s, 9H), 1.40–1.36 (m, 2H), 1.09–0.99 (m, 2H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  170.1, 168.3, 157.7, 156.0, 148.8, 137.2, 129.2, 128.7, 128.3, 127.6, 123.1, 115.7, 113.3, 104.1, 81.0, 65.1, 57.4, 55.0, 29.3, 28.3, 27.8, 18.5. HRMS ( $m/z$ ) calcd. for

$C_{28}H_{34}N_2O_7Na$   $[M + Na]^+$ : 533.2258, found: 533.2255. HPLC purity (TFA): >93.4%. Mp 53–55 °C. IR (neat):  $\nu$  2931, 1692 (strong), 1633, 1546 (strong), 1517, 1367, 1240, 1152, 1104, 834  $cm^{-1}$ .

**(5S)-5-((S)-1-tert-Butoxyethyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.74)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Thr(*t*Bu)-OH (**2.35**) and 4-chloroanisole (**2.53**) as coupling partners, afforded the product as a pale, brown solid (253 mg, 62%).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  10.7 (s, 1H), 7.75 (d,  $J$  = 8.8 Hz, 2H), 6.93 (d,  $J$  = 9.1 Hz, 2H), 4.47 (d,  $J$  = 3.5 Hz, 1H), 4.36 4.31 (m, 1H), 3.75 (s, 3H), 1.49 (s, 9H), 1.17 (s, 9H), 1.00 (d,  $J$  = 6.6 Hz, 3H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  169.1, 168.3, 157.7, 149.2, 128.6, 122.9, 113.4, 105.1, 81.5, 74.7, 66.4, 60.8, 55.0, 27.9, 27.7, 17.8. HRMS ( $m/z$ ) calcd. for  $C_{44}H_{62}N_2O_{12}Na$   $[2M + Na]^+$ : 833.4194, found: 833.4223. HPLC purity (TFA): >94.6%. Mp 152–156 °C. IR (neat):  $\nu$  2977, 2932, 1742, 1702, 1680, 1609, 1515, 1369, 1328 (strong), 1251, 1160 (strong), 1136, 1069, 1031, 983, 810  $cm^{-1}$ .

**(5S)-5-(2-tert-Butoxy-2-oxoethyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.75)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Asp(*t*Bu)-OH (**2.36**) and 4-chloroanisole (**2.53**) as coupling partners and 16 hours reaction time, afforded the product as a brown solid (187, 45%).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  12.0 (broad s, 1H), 7.73 (d,  $J$  = 9.1 Hz, 2H), 6.93 (d,  $J$  = 8.8 Hz, 2H), 4.63 (dd,  $J$  = 5.4, 2.7 Hz, 1H), 3.75 (s, 3H), 3.04 (dd,  $J$  = 14.2, 5.6 Hz, 1H), 2.87 (dd,  $J$  = 13.9, 2.8 Hz, 1H), 1.49 (s, 9H), 1.24 (s, 9H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  168.8, 167.8, 167.6, 157.6, 148.6, 128.6, 123.0, 113.2, 104.0, 81.1, 80.0, 54.9 (two peaks), 35.8, 27.7, 27.3. HRMS ( $m/z$ ) calcd. for  $C_{44}H_{58}N_2O_{14}Na$   $[2M + Na]^+$ : 861.3780, found: 861.3815. HPLC purity (TFA): >95.4%. Mp 127–131 °C. IR (neat):  $\nu$  2975, 2929, 1750 (strong), 1727, 1640, 1608, 1515, 1390, 1351 (strong), 1283, 1242, 1155, 1097, 837, 584  $cm^{-1}$ .

**(5S)-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydro-pyrrol-2-one (2.76)**

Following the general synthesis of  $\alpha$ -aryl tetramic acids (**E**) with Boc-*py*Gly-OH (**2.37**) and 4-chloroanisole (**2.53**) as coupling partners and 16 hours reaction time, afforded the product as a pale, brown solid (85 mg, 28%). Purification required up to 20% MeOH in EtOAc to eluate the product completely.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  12.16 (s, 1H), 7.80 (d,  $J$  = 9.1 Hz, 2H), 6.92 (d,  $J$  = 8.8 Hz, 2H), 4.24 (s, 2H), 3.75 (s, 3H), 1.48 (s, 9H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  168.1, 167.9, 157.5, 149.0, 128.3, 123.5, 113.3, 103.4, 81.0, 55.0, 47.8, 27.8. HRMS ( $m/z$ ) calcd. for  $C_{16}H_{19}NO_5Na$   $[M + Na]^+$ : 328.1155, found: 328.1164. HPLC purity (TFA): >72.0%. Mp  $\approx$  250 °C (decomp.). IR (neat):  $\nu$  2980, 2924, 2853, 1745 (strong), 1635, 1610, 1513, 1430, 1393, 1350, 1289, 1247,

1160, 1093, 984, 858, 797, 750, 578 cm<sup>-1</sup>.

**Attempted utilization of aryldiazonium salts for the  $\alpha$ -arylation of cyclopentane-1,3-dione (**2.77**) for the synthesis of **2.79****

A solution of PdCl<sub>2</sub> (2 mg/mL) was prepared prior to use by suspending the catalyst in MeCN at room temperature and stirred overnight. This resulted in a clear, orange solution of the active catalyst (Pd(MeCN)<sub>2</sub>Cl<sub>2</sub>). Cyclopentane-1,3-dione (**2.77**) (49 mg, 0.50 mmol, 1.00 equiv) and 4-bromobenzenediazonium tetrafluoroborate (**2.78**) (135 mg, 0.50 mmol, 1.00 equiv) were dissolved in MeCN (750  $\mu$ L) and H<sub>2</sub>O (650  $\mu$ L). In some experiments 8% aqueous H<sub>2</sub>SO<sub>4</sub> (650  $\mu$ L) was added instead of H<sub>2</sub>O and in other experiments NaNO<sub>2</sub> (3 mg, 0.05 mmol, 0.10 equiv) was added at this point. While stirring, the Pd(MeCN)<sub>2</sub>Cl<sub>2</sub> solution was added (2 mL). For the experiment employing Pd/C, the catalyst (10 mg) was added under a nitrogen atmosphere in MeOH (2.75 mL) instead of the above-mentioned solvent system. Pd(OAc)<sub>2</sub> was also used directly in the solvent (MeOH or EtOH). The reaction was stirred at room temperature (or heated to 50 °C) overnight and periodically monitored by TLC (EtOAc:MeOH (1:1), KMnO<sub>4</sub> development), LC-MS and <sup>1</sup>H NMR. The crude product was transferred to a separatory funnel with EtOAc (10 mL) and washed with brine (2  $\times$  10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo* affording a colorful/black solid. Analysis by LC-MS and <sup>1</sup>H NMR revealed that no product was formed. Only minor to moderate amounts of the corresponding hydrazone/diazene **2.80**, as well as starting materials, were found.

**3-Amino-4-phenylbutan-2-one hydrochloride (**2.83**)**

Boc-*py*Phe-OH (**2.4**) (200 mg, 0.69 mmol, 1.00 equiv) was dissolved in 37% conc. HCl (10 mL) and heated to 100 °C for 4 hours, at which LC-MS showed full conversion (>95%). The crude product was evaporated to dryness *in vacuo*, the residue was co-evaporated with <sup>i</sup>PrOH (3  $\times$  20 mL) and dried overnight in vacuum to a brown solid (135 mg, 98%), which was subsequently washed several times with cold MeCN to remove (colored) impurities. The hydrochloride **2.83** was obtained as a off-white solid (56 mg, 40%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  8.32 (broad s, 3H), 7.39–7.27 (m, 5H), 4.41 (t, *J* = 6.8 Hz, 1H), 3.21–3.14 (m, 1H), 3.13–3.06 (m, 1H), 2.16 (s, 3H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  204.1, 134.9, 129.5, 128.7, 127.3, 59.2, 35.0, 27.7. MS (*m/z*) calcd. for C<sub>10</sub>H<sub>14</sub>NO [M + H]<sup>+</sup>: 164.1, found: 164.2.

**Attempted ring opening of  $\alpha$ -aryl tetramic acids with conc. HCl for the synthesis of **2.84**, **2.85** and **2.86****

The  $\alpha$ -aryl tetramic acid **2.40** (200 mg, 0.51 mmol, 1.00 equiv) was dissolved in 37% conc. HCl (10 mL) and heated to 100 °C for 4 hours, at which LC-MS showed no traces of the desired product **2.84**. Only the expected Boc-deprotection had happen. The reaction was therefore stirred at reflux overnight. LC-MS showed almost no conversion

of the deprotected  $\alpha$ -aryl tetramic acid, but traces of the corresponding product which has lost the methyl-group, 3-amino-1-(4-hydroxyphenyl)-4-phenylbutan-2-one, was seen. MS ( $m/z$ ) calcd. for  $C_{16}H_{18}NO_2$   $[M + H]^+$ : 256.1, found: 256.1. Similar reactivity was found for **2.57** and **2.66** for the synthesis of **2.85** or **2.86**, respectively.

#### **Attempted synthesis of ethyl enol ether **2.87** from the $\alpha$ -aryl tetramic acid **2.57****

A round-bottomed flask was charged with the  $\alpha$ -aryl tetramic acid **2.57** (23 mg, 0.06 mmol, 1.00 equiv) and an inert atmosphere was introduced by evacuation and back-filling with nitrogen three times. Through a septum, dry THF (1.0 mL) was added and the solution was cooled to 0 °C while stirring. KHMDS (0.5M solution in toluene) (120  $\mu$ L, 13 mg, 0.07 mmol, 1.03 equiv) was added dropwise, and the reaction mixture was stirred at 0 °C for 10 minutes. Then, EtOTs (15 mg, 0.08 mmol, 1.20 equiv) and 18-crown-6 (18 mg, 0.07 mmol, 1.07 equiv) were added as a solution in THF (0.2 mL) and the reaction was aged by slowly heating to ambient temperature during the course of 1 hour. The crude product mixture was analyzed by LC-MS, but no traces of **2.87** were observed. Only the starting material **2.57** was present (with reagents) in the mixture.

#### **Attempted reduction of $\alpha$ -aryl tetramic acids for the synthesis of the $\beta$ -hydroxy derivative **2.88** with a variety of reducing agents**

$\alpha$ -Aryl tetramic acid (**2.57**) (50 mg, 0.14 mmol, 1.00 equiv) was dissolved in a solvent (700  $\mu$ L; DCM, THF or MeOH) and eventually glacial AcOH (70  $\mu$ L) was added. The mixture was cooled to 0 °C and the reducing agent (0.28 mmol, 2.00 equiv;  $NaBH_4$ , DIBAL-H, L-selectride,  $LiAl(O^tBu)_3H$ ,  $LiBH_4$  or 9-BBN) was added portionwise. Bubbles were observed when  $NaBH_4$  and AcOH were used in combination. In some experiments the reducing agent was added at -78 °C and then heated to ambient temperature. The mixture was heated to reflux in other experiments after addition of the reducing agent. After a couple of hours of stirring, water was added to quench the reaction and the organic layer was separated and evaporated to dryness *in vacuo*. Analysis by LC-MS revealed that no reaction had happen and only the starting material **2.57** was recovered. The two other  $\alpha$ -aryl tetramic acids **2.40** and **2.66** were also subjected to a variety of reducing agents, but the same unreactivity was observed.

**Attempted ring opening of 2.57 with LiOOH for the synthesis of the  $\beta$ -keto acid 2.89**

In a mixture of THF (4.0 mL) and H<sub>2</sub>O (1.3 mL) was added the  $\alpha$ -aryl tetramic acid **2.57** (185 mg, 0.51 mmol, 1.00 equiv) and the solution was cooled to 0 °C. LiOH (30 mg, 1.26 mmol, 2.50 equiv) and 35% H<sub>2</sub>O<sub>2</sub> (0.25 mL) were added. The reaction mixture was stirred at 0 °C for 30 minutes and then slowly heated to ambient temperature. Quenched by addition of sat. aqueous Na<sub>2</sub>SO<sub>3</sub> (10 mL) and diluted with brine (10 mL). Extracted with EtOAc (3  $\times$  15 mL), acidified with 2M HCl to pH 2 and then extracted with EtOAc again (3  $\times$  15 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. The crude product was analyzed by LC-MS, which showed no product formation at all and only recovery of **2.57**.

**Attempted ring opening of 2.57 with Sml<sub>2</sub> for the synthesis of the  $\beta$ -keto aldehyde 2.90**

A nitrogen-filled flask was charged with the  $\alpha$ -aryl tetramic acid **2.57** (21 mg, 0.06 mmol, 1.00 equiv) and dry THF (2 mL) was added. While stirring at room temperature was added Sml<sub>2</sub> (0.1M in THF) (1.15 mL, 46 mg, 0.12 mmol, 2.00 equiv) quickly in one portion. The reaction mixture became immediately blackish in color. After 5 minutes of stirring, 99.9% EtOH (10  $\mu$ L, 5 mg, 0.12 mmol, 2.00 equiv) was added and the reaction was stirred for 1 hour. LC-MS analysis of the mixture revealed no product formation and only the starting material **2.57** was seen.



## Chapter 3

# Synthesis and biological activity of FVIIa inhibitors

### 3.1 Synthesis of C-terminal modified peptides

Synthesis of peptides is usually performed using a standardized Fmoc solid-phase approach.<sup>328</sup> However, synthesis of C-terminal modified peptides is less straightforward. This introduction will therefore cover different possible synthetic strategies; solution-phase, polymer-assisted and solid-phase peptide synthesis.

#### 3.1.1 Solution-phase peptide synthesis

It is reasonable to consider a solution-phase synthesis of small peptides, because the number of coupling/deprotection steps is limited. If the selected C-terminal modification can resist these conditions, a solution-phase strategy is probably the most straightforward for a rapid preparation of a variety of small modified peptides. Formation of peptide bonds is a well known process and hence the optimization should hopefully be relatively easy.

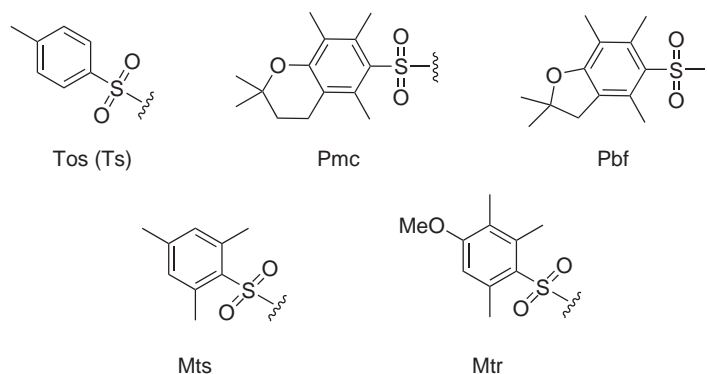
Boc/Bn-amino acids are usually the building blocks of choice for solution-phase peptide synthesis. However, other protection groups exist and may be considered for optimal orthogonal  $N^\alpha$ -deprotection during the peptide synthesis. Although Fmoc-groups are easily removed by treatment with 20% piperidine in DMF,<sup>328</sup> the fulvene-piperidine adduct is difficult to remove in solution-phase and may lead to unwanted by-products. Several methods have been reported as improved removal conditions in solution-phase, e.g. the use of tris(2-aminoethyl)amine,<sup>329</sup>  $\text{AlCl}_3$ /toluene<sup>330</sup> or DBU/1-octanethiol.<sup>331</sup> However, Boc-groups are still easier to deprotect because the by-product is simply removed by evaporation. TFA in DCM are typically the removal conditions of choice, but

conditions such as  $\text{BF}_3\text{-OEt}_2$ ,<sup>332</sup>  $\text{AlCl}_3$ ,<sup>333</sup> 85% aqueous  $\text{H}_3\text{PO}_4$ ,<sup>334</sup>  $\text{Sn}(\text{OTf})_2$ <sup>335</sup> and  $\text{H}_2\text{O}/150\text{ }^\circ\text{C}$ <sup>336</sup> have been reported as milder and more selective reagents. Besides of a  $N^\alpha$ -protection, functionalized amino acids have to be side-chain protected.

Another parameter to consider is the choice of coupling agent. An appropriate agent should be selected to provide high yield and easy purification. Solid-phase peptide synthesis uses coupling agents in excess and purification simply by filtration.<sup>328</sup> This is not possible in the solution-phase and the amount of coupling agent should therefore be kept to a minimum for optimal purification.

### 3.1.1.1 Side-chain protection groups

Arginine is  $N^\omega$ -protected to prevent deguanidination<sup>337</sup> and  $\delta$ -lactam formation as a result of the nucleophilicity of the guanidino group.<sup>338</sup> On the other hand, deprotection of guanidino groups is rather difficult and due to their basicity ( $\text{p}K_a = 12.5$ ), they are protonated almost entirely throughout the peptide synthesis.<sup>339,340</sup> In principle, protection of all nitrogen atoms would be required to fully mask its nucleophilicity, but practically only one or two of them is protected.

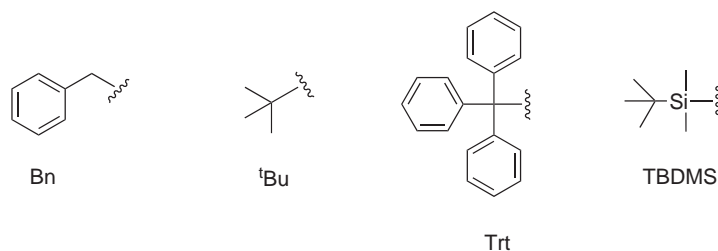


**Figure 3.1.1:** A variety of arginine  $N^\omega$ -protection groups used to avoid deguanidination<sup>337</sup> and  $\delta$ -lactam formation during peptide synthesis<sup>338</sup>

The most widely used  $N^\omega$ -protecting for the Boc/Bn-strategy is Tos,<sup>341</sup> whereas for the Fmoc/ $t$ Bu strategy Pbf or Pmc<sup>338</sup> is usually used (see Figure 3.1.1). Tos is removed by harsh conditions such as HF, TFMSA-TFA-thioanisole or  $\text{Na}/\text{NH}_3$ .<sup>342</sup> Pmc and Pbf are much easier to remove, simply by treatment with TFA.<sup>321,343</sup> Pbf is more acid-labile than Pmc and quite cheaper.<sup>344</sup> During deprotection of the Pmc/Pbf-group there is a risk of migration and hence a sulfonation of tryptophan, serine and threonine residues.<sup>345</sup>



However, this migration can be minimized by addition of suitable scavengers.<sup>346</sup> Other common  $N^\omega$ -protection groups are Mts (removed by TFMSA-TFA-thioanisole),<sup>347,348</sup> Mtr (TFA-thioanisole),<sup>349</sup>  $-\text{NO}_2$  (HF or  $\text{H}_2$ -Pd/C)<sup>350</sup> and Cbz<sub>2</sub> ( $\text{H}_2$ -Pd/C)<sup>351</sup> with removal conditions stated in brackets (see Figure 3.1.1).



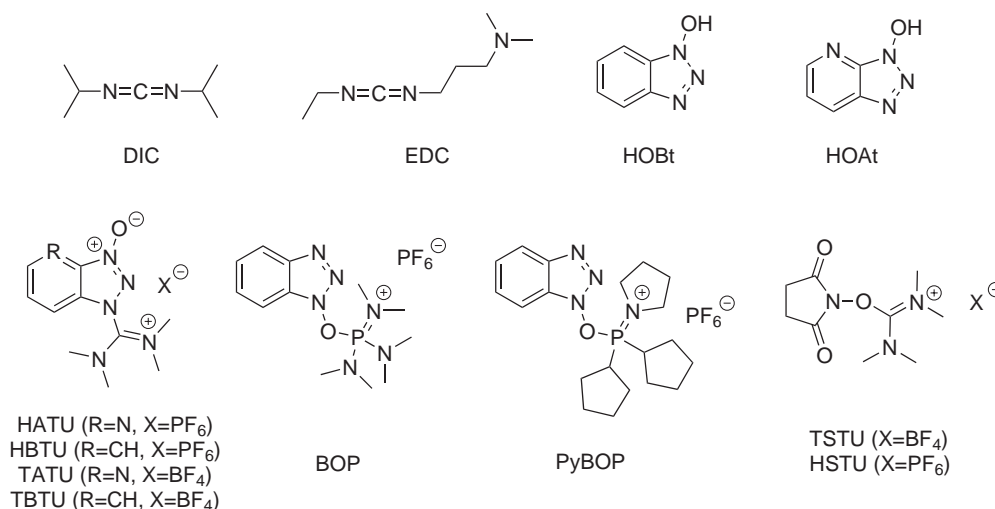
**Figure 3.1.2:** Common threonine and tyrosine *O*-protecting groups used to avoid *O*-acylation and other side reactions.<sup>338</sup>

Threonine is *O*-protected to avoid dehydration and *O*-acylation possibly followed by *O*-*N* migration.<sup>338</sup> Protection of the hydroxy group is in fact more important in solid-phase peptide synthesis, because an excess of coupling agent is used. Coupling with unprotected threonine has been reported for several syntheses, including solid-supported approaches.<sup>218,352</sup> The *O*-protection is typically introduced as ethers because they are more stable than carbamates or esters. The most widely used protection group for the Boc/Bn strategy is a Bn-protection (see Figure 3.1.2), which is removed with HF<sup>353</sup> or by hydrogenolysis catalyzed by Pd/C.<sup>354</sup> A catalyst combination of Pd/C and Pd(OH)<sub>2</sub>/C (Pearlman's catalyst) in equal amounts has been reported as a more efficient catalytical system for *O*-debenzylation,<sup>355</sup> because Pd(OH)<sub>2</sub>/C turned out to be more active than Pd/C.<sup>356</sup> Selection of appropriate deprotection conditions may require screening of several different catalysts, solvents and concentrations.<sup>356</sup> A variety of Lewis acids can also be used for *O*-debenzylation, e.g. TiCl<sub>4</sub> and SnCl<sub>4</sub>.<sup>357</sup>

Use of unprotected tyrosine can also lead to *O*-acylation because of the nucleophilicity of the phenolate ion under basic conditions as well as *ortho*-alkylation because of the electron-rich aromatic ring.<sup>338</sup> The most commonly used *O*-protections of tyrosine are the Bn- and *t*Bu-groups, which are removed by catalytic hydrogenolysis<sup>354</sup> or TFA,<sup>358</sup> respectively. Protecting groups such as Cbz (removed with HF),<sup>359</sup> Boc (TFA)<sup>360</sup> and TBDMS (TBAF)<sup>361</sup> have also been used as *O*-protection of tyrosine, with deprotection conditions stated in brackets (see Figure 3.1.2).

### 3.1.1.2 Coupling agents

Formation of peptide bonds is performed using a coupling agent, which can be selected among hundreds of different compounds.<sup>362</sup> The most commonly used agents are carbodiimides (e.g. DIC<sup>363</sup> or EDC<sup>364</sup>), 1*H*-benzotriazole-based uronium/aminium salts (e.g. HATU,<sup>365,366</sup> HBTU,<sup>365–367</sup> TATU<sup>368</sup> and TBTU<sup>369</sup>), phosphonium salts (e.g. BOP<sup>370</sup> and PyBOP<sup>371</sup>) or 2-hydroxysuccinimide-based coupling agents (e.g. TSTU<sup>369</sup> or HSTU<sup>372</sup>) (see Figure 3.1.3).



due to the carcinogenicity and respiratory toxicity associated with hexamethylphosphoramide,<sup>362</sup> and therefore PyBOP was developed<sup>371</sup> and highly recommended as a substitute. TSTU and HSTU are one of the few coupling reagents incorporating another system than HOAt/HOBt. These agents are based on the hydroxysuccinimide system,<sup>369,372</sup> and hence also have to be used in combination with an epimerization-suppressing additive.<sup>369</sup>

Selecting appropriate coupling reagents may be difficult. However, the differences between the many reagents are small. In general DIC/HOBt or HOAt remains an excellent choice for many substrates, and selecting suitable coupling reagents should be kept simple and 'exotic' reagents should be avoided.<sup>362</sup> HOAt as an additive is superior to HOBt for stepwise/linear peptide synthesis with respect to reaction rate and degree of racemization.<sup>376</sup>

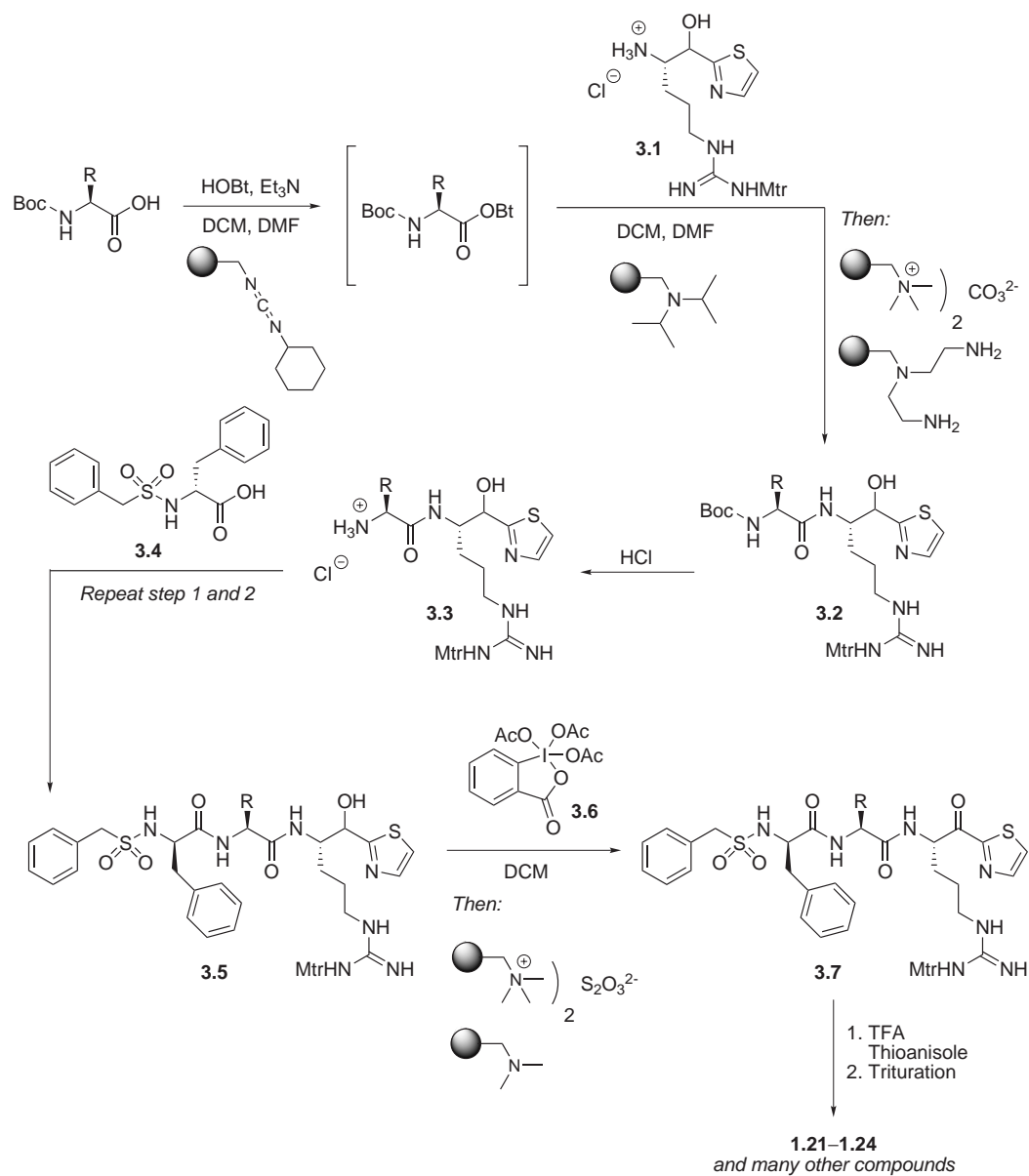
Several coupling agent systems have been reported as especially appropriate for solution-phase synthesis of peptides, e.g. DCC/HOBt or HONb,<sup>377</sup> EDC/HOBt<sup>378,379</sup> and PyBOP.<sup>380</sup> EDC is slightly cheaper than PyBOP, but compared to DIC and DCC both of these coupling agents are quite expensive.<sup>251</sup>

### 3.1.2 Polymer-assisted solution-phase peptide synthesis

The advantage of solid-phase peptide synthesis is the possibility to use coupling agents in excess, which secures successful coupling with less reactive amino acids/peptides. Moreover, the purification is very simple, because the reagents can be removed by a simple filtration.<sup>381</sup> However, when the C-terminal is modified, standard methods for anchoring to the solid support cannot be used. As an alternative, polymer-assisted reagents can be used, e.g. immobilized coupling agents and bases.

A library of  $\alpha$ -ketothiazole FVIIa inhibitors were synthesized using polymer-assisted solution-phase peptide synthesis from the reduced building block **3.1** (see Scheme 3.1.1).<sup>165,166</sup> These inhibitors (**1.21–1.24**) were presented in Chapter 1 (c.f. Figure 1.1.22). The first peptide coupling afforded the dipeptide **3.2** using immobilized cyclohexyl carbodiimide and diisopropyl amine. Purification was accomplished by using immobilized trimethylammonium carbonate and di(2-aminoethyl)amine. Then a  $N^\alpha$ -deprotection afforded the free amine **3.3** and subsequently another peptide coupling was carried out with  $N$ -(benzylsulfonyl)-D-phenylalanine **3.4**, giving the  $N$ -terminal modified tripeptide **3.5**, which was converted into the desired  $N^\omega$ -protected  $\alpha$ -ketothiazole **3.7** upon oxidation with the periodinane Dess-Martin reagent **3.6**. Once again immobilized bases were used for purification. A deprotection with TFA/thioanisole afforded the target peptides. Overall yields ranged from 9 to 45% based on mass recovery and purity

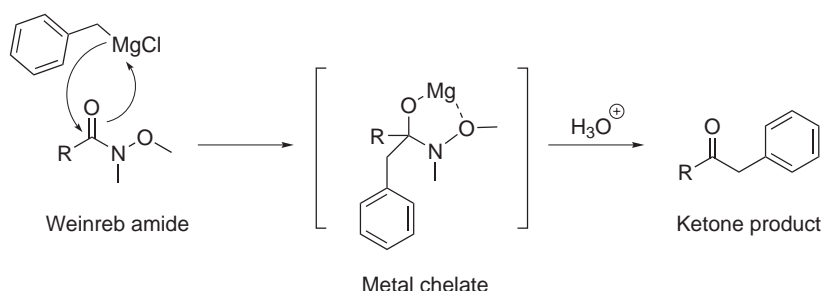
was 82% on average.<sup>165,166</sup>



**Scheme 3.1.1:** Polymer-assisted solution-phase synthesis of  $\alpha$ -ketothiazoles as FVIIa peptidyl inhibitors.<sup>165,166</sup>

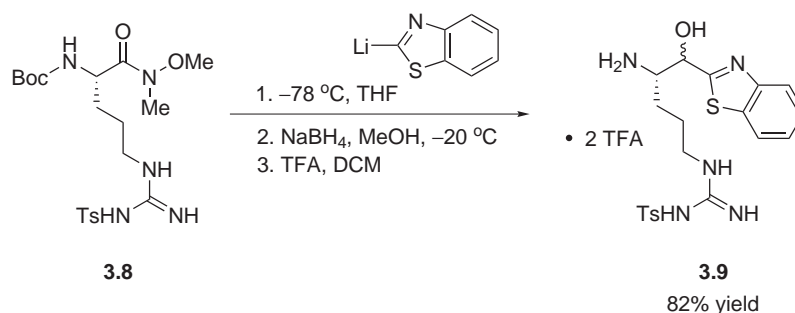
The  $\alpha$ -heterocyclic building block can be synthesized in different ways,<sup>382</sup> but the Weinreb amide strategy is one of the most general ones. Usage of Weinreb amides was introduced in 1981 to avoid overaddition of an organometallic reagent giving the unde-

sired tertiary alcohol.<sup>383</sup> Overaddition is not observed because a stable metal-chelate is formed upon addition of the first equiv (see Scheme 3.1.2 for a general example).<sup>383</sup> Upon hydrolysis the chelate is decomposed affording the desired ketone product.



**Scheme 3.1.2:** Weinreb amides are easily converted into ketones upon addition of e.g. a Grignard reagent. Only one equivalent is added due to formation of a stable chelate.<sup>383</sup> Here is shown a general example.

Traditionally,  $\alpha$ -amino ketones have been synthesized from the corresponding Weinreb amide by addition of a Grignard<sup>193,384–387</sup> or a lithium reagent.<sup>388</sup> Unfortunately, the method requires an excess of the organometallic reagent, because the amino acid contains at least one exchangeable proton ( $N^{\alpha}$ -H). If the organometallic reagent is expensive and not commercially available, pre-deprotonation can be achieved by addition of 1 equiv of a simple alkyl Grignard base, such as  $i$ PrMgCl or MeMgCl.<sup>389</sup>



**Scheme 3.1.3:** Synthesis of the  $\alpha$ -ketobenzothiazole **3.9** via the Weinreb amide **3.8** followed by reduction and N-deprotection.<sup>104</sup>

By this procedure, the  $\alpha$ -ketone benzothiazole building block **3.9** was synthesized by addition of a metalated heterocycle to the Weinreb amide **3.8** in good yield (see Scheme

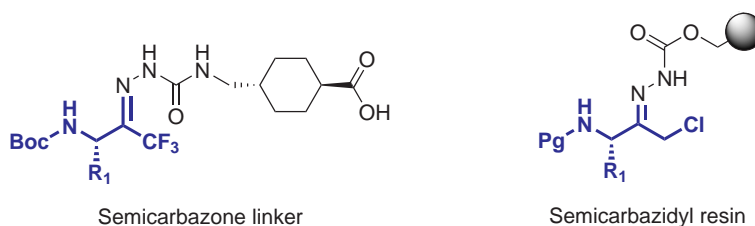
3.1.3).<sup>104</sup> The ketone formed, even though it is desired in the final product, was reduced to the secondary alcohol by  $\text{NaBH}_4$  to avoid epimerization of the  $\alpha$ -stereocenter during peptide synthesis and to avoid unwanted reactions.<sup>382</sup> A similar approach was used for the preparation of the reduced  $\alpha$ -ketothiazole building block **3.1** (c.f. Scheme 3.1.1).<sup>165,166</sup> Due to the electron deficiency of the carbonyl group in these inhibitors, epimerization of the adjacent stereogenic center in the final inhibitor is rapidly occurring in whole blood<sup>390</sup> or under mild alkaline aqueous conditions.<sup>104</sup> This demonstrates why the reduced form of the building block is used during synthesis of these very electron deficient ketones. However, no reports in the literature states if this pronounced epimerization causes any decrease in biological activity *in vitro* or *in vivo*.

### 3.1.3 Solid-phase peptide synthesis

Solid-phase syntheses of C-terminal modified peptides have been described in the literature,<sup>391</sup> by choosing a resin/linker depending on the desired C-terminal functionality. Solid-phase procedures for the synthesis of such peptides are complicated due to the fact that the C-terminal residue is generally attached to the resin through its  $\alpha$ -carboxyl group in the most commonly used  $C \rightarrow N$  synthetic approach.<sup>391</sup> Two alternative strategies have therefore been used for solid-phase synthesis of C-terminal modified peptides; *i*) direct synthesis involving the use of specialized linkers or resins that are appropriately designed to allow anchoring of the C-terminal and to generate a specific end group upon cleavage, or *ii*) methods of general applicability regardless of the C-terminal functional group present, e.g. side-chain or backbone amide lining (BAL) as well as inverse solid-phase peptide synthesis.<sup>391</sup> The latter is a non-traditional  $N \rightarrow C$  directional approach,<sup>392–395</sup> which has only been reported briefly in the literature primarily because of low yields and high degree of racemization.<sup>391</sup>

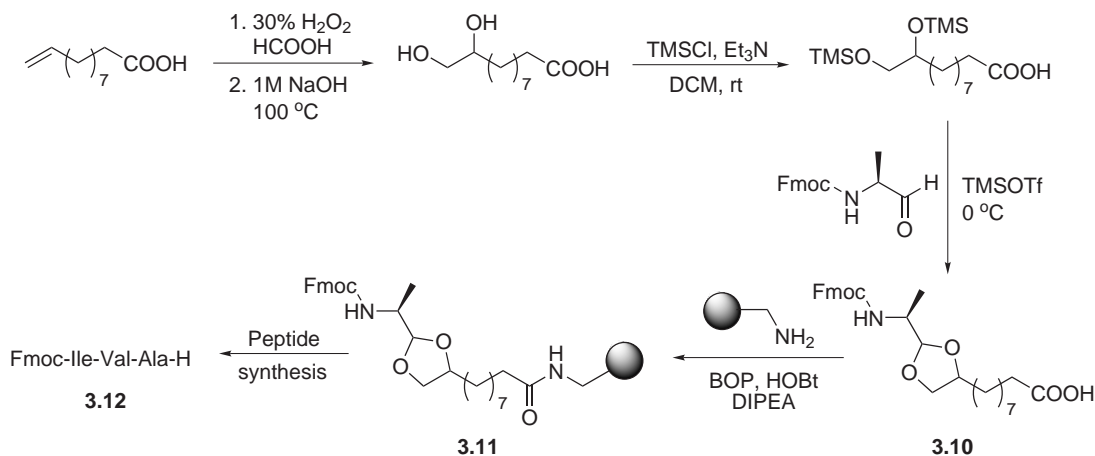
#### 3.1.3.1 C-Terminal carbonyl anchoring via specialized linkers

Only a few linkers/resins have been reported for the direct synthesis of C-terminal modified peptide ketones, as most of the examples afford aldehydes (for example via a thiazolidine linker,<sup>396</sup> oxazolidine<sup>397,398</sup> or a Wittig resin<sup>399–401</sup>). However, synthesis of ketone C-terminal modified peptides has been reported using a semicarbazone linker<sup>402–404</sup> for the preparation of trifluoromethyl ketones or a semicarbazidyl resin<sup>405</sup> for the preparation of chloromethyl ketones (see Figure 3.1.4). The products are released from the solid-support by treatment with TFA.<sup>405</sup> A variety of cysteine protease inhibitors was synthesized in this way.<sup>405</sup>



**Figure 3.1.4:** The semicarbazone linker and the semicarbazidyl resin enable the anchoring of the C-terminal carbonyl group to a solid support.<sup>402–405</sup>

Carbonyl groups can also be anchored to a solid support through acetal formation. For example, the peptide aldehyde **3.12** was synthesized in this way. At first, the *O*-protected DHUA linker (10,11-dihydroxy-undecanoic acid) was synthesized in three steps from undec-10-enoic acid (see Scheme 3.1.4). The P1-amino acid-derived aldehyde was anchored to the linker mediated by TMSOTf giving the acetal linker **3.10**, which was subsequently attached to a solid support under traditional coupling conditions giving **3.11**. Classical Fmoc/*t*Bu solid-phase peptide synthesis (BOP, HOBT, DIPEA) afforded the anchored tripeptide acetal, which was released as the peptidyl aldehyde **3.12** upon treatment with 95% TFA in water with no racemization observed<sup>406</sup> (see Scheme 3.1.4).



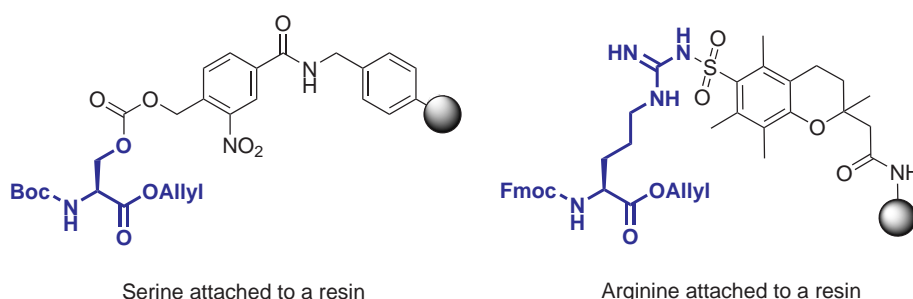
**Scheme 3.1.4:** Synthesis of the DHUA-linker **3.10** and synthesis of the peptide aldehyde **3.12** through classical Fmoc/*t*Bu solid-phase peptide synthesis.<sup>406</sup>

This approach is very cost-effective and does not involve reduction or oxidation procedures.<sup>406</sup> The utilization of diol linkers for anchoring of carbonyl groups as acetals has also been reported in connection with chemical modifications of terephthaldehydes and

isophthalaldehydes,<sup>407,408</sup> solid-supported Suzuki-Miyaura cross-couplings<sup>409</sup> and synthesis of a steroidal ketone derivative,<sup>410</sup> demonstrating different linker-coupling methods, such as azeotropic reflux and acetal exchange catalyzed by  $\text{Sc}(\text{OTf})_3$ .<sup>410</sup>

### 3.1.3.2 Side-chain linking

Side-chain attachment does not involve the C-terminal carboxyl group, which allows considerably flexibility in terms of modification of the terminal and an easy and direct preparation of peptides containing a variety of C-terminal modifications can be accomplished (see Figure 3.1.5).



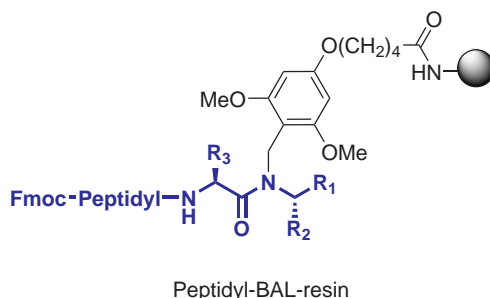
**Figure 3.1.5:** Serine and arginine are two amino acids that can be anchored to a solid support through the side-chain functionality.<sup>411–415</sup>

However, the general applicability of this approach is clearly limited to relatively few trifunctional amino acid residues that are suitable positioned in the peptidyl sequence.<sup>391</sup> Anchoring the side-chains of aspartic and glutamic acid,<sup>416</sup> lysine and ornithine,<sup>417</sup> serine,<sup>411,412</sup> threonine,<sup>412</sup> tyrosine<sup>418,419</sup> and arginine<sup>413–415</sup> have been reported (see Figure 3.1.5). For example, serine can be anchored to a carboxyloxymethyl Nbb resin, which can be cleaved from the solid support upon  $h\nu$  (350 nm) radiation,<sup>411</sup> whereas arginine can be anchored through a Pmc-like linker and released with TFA.<sup>415</sup>

### 3.1.3.3 Backbone amide linking (BAL)

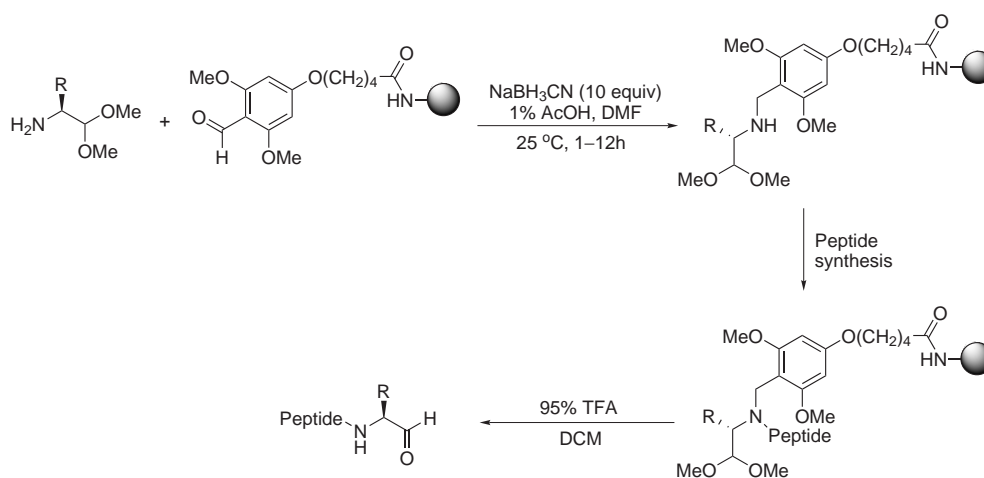
Anchoring the backbone amide is better known as the BAL-strategy. It is usually based on the 2,4,6-trisalkoxybenzyl amide linker (PAL) system<sup>420,421</sup> (see Figure 3.1.6), but other systems have also been used for construction of BAL-resins, such as 2-nitrobenzylamide,<sup>422</sup> 4-alkoxybenzylamide,<sup>423</sup> and the 3-indole system.<sup>424</sup>





**Figure 3.1.6:** Peptide anchored to a solid support through the backbone amide based on the 2,4,6-trisalkoxybenzyl amide linker (PAL) system.<sup>420,421</sup>

The approach using a BAL-resin has been used for preparation of C-terminal peptide aldehydes by anchoring amino acetals to PALdehyde-PS by  $\text{NaBH}_3\text{CN}$ -promoted reductive amination. After appropriate peptide synthesis, the peptide was cleaved from the linker upon treatment with 95% TFA (see Scheme 3.1.5). These conditions also hydrolyze the acetal hence forming the desired C-terminal aldehyde.<sup>425</sup>



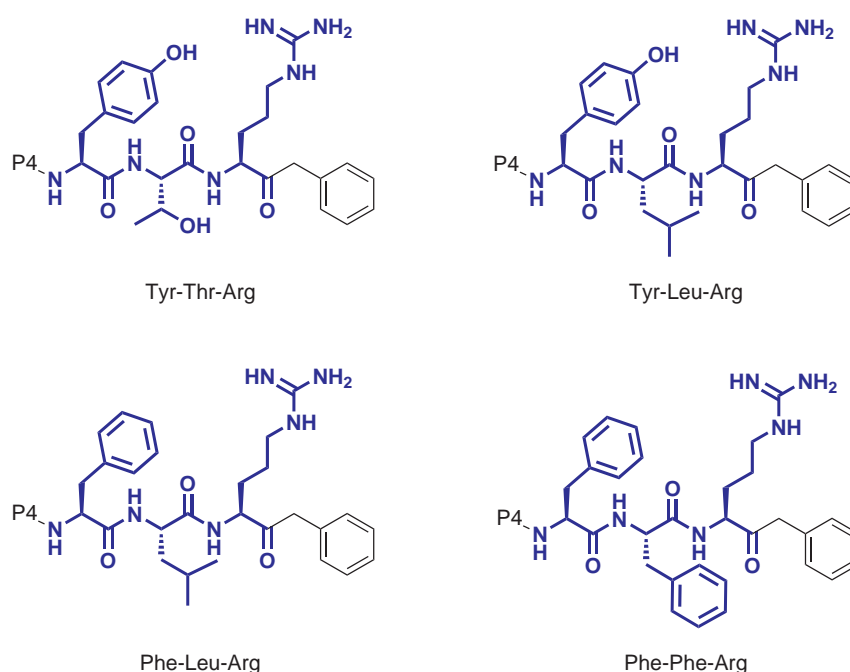
**Scheme 3.1.5:** Synthesis of peptidyl aldehydes using the backbone amide linking (BAL) approach for solid-phase peptide synthesis.<sup>425</sup>

In general, two steps need to be carefully considered when using the BAL-strategy. Primarily, an optimized procedure for on-resin reductive amination involving mixing a resin-bound aldehyde, an amino acid derivative, and  $\text{NaBH}_3\text{CN}$  simultaneously. Under these neutral to slightly acidic conditions, the reaction should proceed to completion without any detectable racemization. Secondly, acylation of the resulting secondary  $\alpha$ -amine attached to the BAL-resin is a more challenging step compared to acylations of unsubstituted primary amines.<sup>391</sup>

## 3.2 Results and discussion

### 3.2.1 Design of peptidyl benzyl ketone FVIIa inhibitors

Based on the FVIIa specificity profile<sup>168</sup> presented in Section 1.1.4, four different tripeptide sequences were selected (see Figure 3.2.1). These sequences were chosen among the most active amino acids in each of the three positions, P3-P1. Arginine was significantly more active in the P1-position than any other amino acid,<sup>168</sup> and therefore all peptides will contain an arginine at that position.



**Figure 3.2.1:** Selected tripeptide sequences chosen from the FVIIa specificity profile<sup>168</sup> among the most active amino acids in each of the three positions, P3-P1.

Moreover, a small variety of different *N*-terminals will be tested; a free *N*-terminal (P4 = H), an acetyl group (P4 = Ac) or a benzyloxycarbonyl group (P4 = BnO<sub>2</sub>C). The free *N*-terminal would be the most convenient, because no further modification is needed and the peptide can be obtained by a simple deprotection. However, since FVIIa is an endopeptidase, inhibitors with a free *N*-terminal is most likely not the most active ones. Acetylation of the *N*-terminal is a simple way for capping the amino group, and therefore this group will be briefly investigated. In the literature, there is precedence for that a *N*-BnO<sub>2</sub>-terminal would be an appropriate choice,<sup>130,165,166</sup> and therefore this

group is included as one of the possible *N*-terminals. The benzyloxy carbamate group (P4 = Cbz) will be tested as an alternative to the BnSO<sub>2</sub>-group, because Cbz can be conveniently introduced directly with the P3-amino acid.

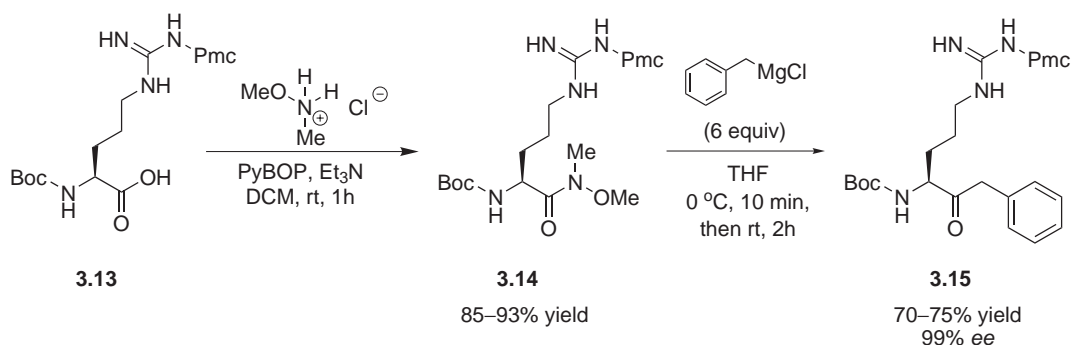
Many of the reported inhibitors have a D-amino acid in the P3 position, but unnatural configurations were not investigated in the FVIIa specificity profile.<sup>168</sup> In the literature it is not stated if the usage of D-amino acids is to increase the *in vivo* stability (D-amino acids are not recognized by proteases/peptidases) or to increase the activity of the inhibitor. Therefore, some of the peptides in this project will be prepared and tested with a D-amino acid in the P3-position.

An unsubstituted benzyl ketone (P1') will be used and no further functionalization will be investigated at this point. Mainly this is because a mild and rapid synthetic route of the building block is still lacking, and thus it has to be synthesized via a Weinreb amide (c.f. Section 3.2.2) using excesses of a Grignard reagent. Moreover, the simple building block has been selected to limit the number of peptides to be synthesized. Eventually, functionalization of the benzyl ketone would be of great interest; as a probe for the S1' binding pocket, but also for a potential alteration of the electrophilicity of the carbonyl group. Consequently, this might alter the potency of the inhibitors. When one or more potent FVIIa inhibitors have been identified by altering the P4-P1 scaffold, a SAR study of the P1' benzyl ketone would be possible using commercially available benzylmagnesium Grignard reagents with substituents such as halides (Cl, Br, F), alkyl (Me, <sup>i</sup>Pr, <sup>t</sup>Bu), alkoxy (OMe, OEt, OBn, OCF<sub>3</sub>) and thioethers (SEt).<sup>426</sup> Many different substitution-patterns are available, including *ortho*-substituted reagents, but functionalities such as ketones, aldehydes, esters and nitrils are of course not available, which is a significant limitation in a medicinal chemistry project.

### 3.2.2 Synthesis of arginyl benzyl ketone building block

In view of the fact that a novel and mild synthesis of the arginyl benzyl ketone building block could not be achieved (c.f. Chapter 2), a traditional method was used. As described above, ketone building blocks have successfully been synthesized via a Weinreb amide.<sup>104,165,166</sup> Thus, the building block **3.15** was prepared in two steps from Boc-Arg(Pmc)-OH (**3.13**) via the Weinreb amide **3.14** (see Scheme 3.2.1).

Boc-Arg(Pmc)-OH (**3.13**) was chosen as the starting material for several reasons. First of all, a Boc-protected amino acid is stable under basic conditions. In fact, attempts with a Fmoc-protection group resulted in its removal and no product was formed during reaction with the benzyl Grignard reagent. Secondly, a *N*<sup>ω</sup>-protection with Pmc was selected, because it is much easier to remove than Tos or NO<sub>2</sub>, and yet less acid-labile



**Scheme 3.2.1:** The arginyl benzyl ketone building block **3.15** was synthesized in two steps from Boc-Arg(Pmc)-OH **3.13** via the Weinreb amide **3.14** in good overall yield and with no racemization.

than Pbf and Mtr, which are also common protection groups of arginine. Boc-Arg(Pmc)-OH (**3.13**) therefore seems to be the most appropriate, commercially available starting material for the building block synthesis.

Formation of the Weinreb amide **3.14** was afforded using PyBOP as coupling agent, inspired by previous results using BOP.<sup>214</sup> This step proceeded smoothly giving rise to the Weinreb amide **3.14** in good yield. Formation of the benzyl ketone **3.15** was accomplished by treating the Weinreb amide **3.14** with excess amounts of benzylmagnesium chloride, initially at 0 °C and then heating to room temperature. It was found, that approx. 6 equiv of the Grignard reagent was necessary for complete conversion of **3.14**. The reaction was rather slow at low temperatures (−78 °C to 0 °C), but when heated to room temperature it proceeded smoothly affording good yield and no racemization as determined by chiral HPLC.

Usage of 6 equiv of this basic nucleophilic reagent indeed demonstrates the drawback of this method for preparation of the arginyl benzyl ketone building block **3.15**. There are no reasons for why such a huge excess is needed, other than that at least 1 equiv is deprotonating the *N*<sup>α</sup>-proton. A commercially available Grignard reagent was used, but neither a new nor a old bottle changed the requirement of such a large excess. However, on laboratory-scale and with a non-functionalized Grignard reagent, this method in fact seems to be the best way for a rapid and easy preparation of the building block **3.15** in gram-scale.

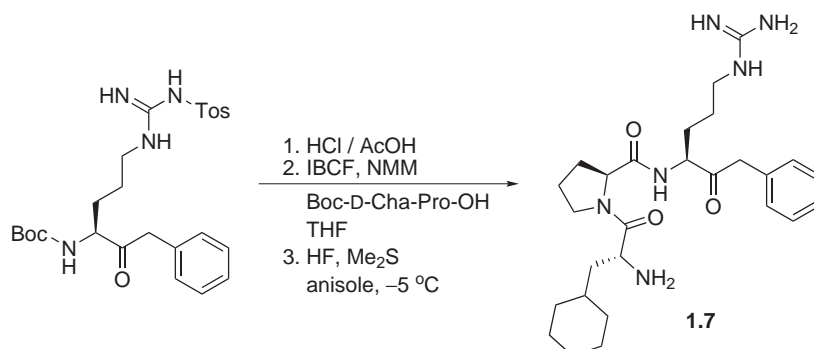
### 3.2.3 Solution-phase C-terminal modified peptide synthesis

Choosing a synthetic strategy is always a balance between advantages and disadvantages. Based on the literature reported on synthesis of C-terminal modified peptides, all three major strategies seem usable for this project. However, a solution-phase synthetic approach is probably the most straightforward, if the building block **3.15** is compatible with the repeating coupling/deprotection conditions. This method uses commercially and readily available reagents, but the purification after each coupling step will be time-consuming because each step needs to be purified individually. On the other hand, peptide couplings are usually easy to form and optimization of the procedure should be very limited giving a rapid access to a variety of interesting FVIIa inhibitors.

Polymer-assisted synthesis would definitely be an improvement of the solution-phase approach, but these reagents are not as readily available as the traditional ones. Using immobilized reagents would most likely improve the purification of each step, but the reactivity might be lower due to insufficient diffusion into the solid-phase and thus require further optimization. It is, however, a rather simple approach to test if a solution-phase method is already established.

Last but not least, a solid-supported method could be used, e.g. by anchoring the ketone as an acetal, the arginine side-chain to a functionalized linker or by using a backbone amide linking (BAL). Once again this method utilizes less readily available reagents and the attachment is not general and may require lengthy optimization procedures. Furthermore, solid-phase chemistry is usually entirely based on the Fmoc  $N^\alpha$ -protection, which is not compatible with the Weinreb-strategy for the building block synthesis. Deviations of these standardized conditions would be rather impractical for a convenient and easy solid-supported synthesis. Using the building block **3.15** would be problematic, because cleavage from the linker/resin is performed by treatment with TFA and acid in particular is the reagent of choice for deprotection of Boc-groups and Pmc/Pbf. Screening of milder and more selective conditions for deprotection of the Boc-group would therefore be necessary. Attaching the carbonyl group to the solid-phase e.g. via a semicarbazidyl resin or via an acetal, would on the other hand serve as a protection of the functionality and avoid by-product formation as a result of reactions with the electrophilic carbonyl. Protection (or reduction) of electron deficient carbonyl functionalities is especially important due to their enhanced electrophilicity.

Based on the potential problems with a solid-supported synthesis it was decided to synthesize the FVIIa inhibitors by solution-phase peptide synthesis. Hereby, a rapid preparation of a range of peptides should be possible. The only previously reported peptidyl benzyl ketone is the thrombin inhibitor H-D-Cha-Pro-Arg-bk (**1.7**) (bk = benzyl ketone),<sup>105</sup> which was described in Section 1.1.3.1. This inhibitor was synthesized by a convergent approach coupling an arginyl benzyl ketone building block with the dipeptide



**Scheme 3.2.2:** The benzyl ketone serine protease inhibitor **1.7** was synthesized by coupling the dipeptide Boc-D-Cha-Pro-OH with the building block. However, the authors do not comment on the high possibility of racemization.<sup>105</sup>

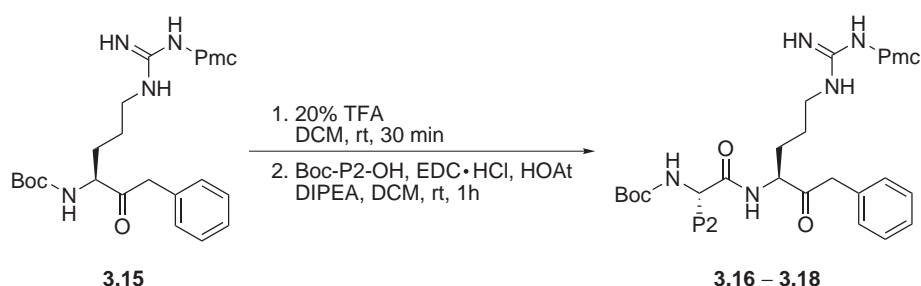
Boc-D-Cha-Pro-OH using IBCF as coupling agent (see Scheme 3.2.2). Convergent synthesis of peptides usually requires specialized resins<sup>427</sup> or other special conditions, such as enzymatic condensation,<sup>428</sup> to avoid racemization. In the case of **1.7**, convergent synthesis is successful because proline is the C-terminal residue. Minimization of the risk of racemization can be achieved by having proline, glycine, alanine or arginine as C-terminal residues.<sup>429</sup> Unfortunately these amino acids are not included in the FVIIa-TF specificity profile at the P2 position. A traditional linear synthetic approach therefore seems to be the most rational choice for preparation of peptidyl benzyl ketone FVIIa inhibitors.

### 3.2.3.1 Synthesis of the P3-P2 scaffold

With the P1-amino acid in hand, the next step was to synthesize a variety of different tripeptides by two consecutive peptide couplings.

Initially, a chemoselective deprotection procedure was investigated to selectively remove the Boc-group in the presence of the Pmc-group. Luckily, this was done easily by treatment with 20% TFA in DCM at room temperature for 30 minutes (see Table 3.2.1). LC-MS showed full conversion and no loss of the Pmc-group was observed.

Next, the peptide coupling was afforded using a Boc-protected amino acid and EDC/HOAt as coupling agents. EDC was chosen over DIC and DCC because the corresponding urea by-product is soluble in acid and therefore enhances the ease of purification. One drawback of using EDC is the price since this carbodiimide is almost 40 times more expensive than e.g. DCC.<sup>251</sup> Using EDC/HOAt, three dipeptides were synthesized (**3.16–3.18**) in

**Table 3.2.1:** *Synthesis of protected dipeptides (P2-P1).*

| entry | P2 <sup>a</sup> | product     | yield (%) <sup>b</sup> | purity (%) <sup>c</sup> |
|-------|-----------------|-------------|------------------------|-------------------------|
| 1     | Thr(Bn)         | <b>3.16</b> | 40–69                  | >96.4                   |
| 2     | Leu             | <b>3.17</b> | 34–62                  | >94.8                   |
| 3     | Phe             | <b>3.18</b> | 46–60                  | >91.7                   |

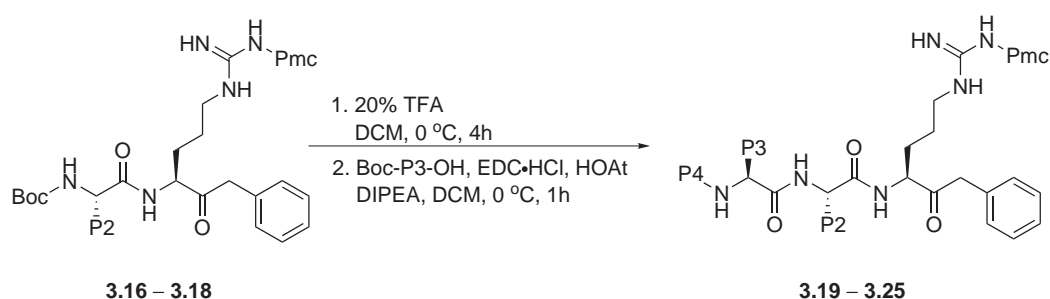
<sup>a</sup>: Notation corresponds to the respective amino acid side-chain. <sup>b</sup>: Purified yield. <sup>c</sup>: The lowest determined purity based on UPLC analysis (TFA).

moderate to good purified yield (34–69%). The dipeptides were easily purified by flash chromatography and subsequently subjected to the next peptide coupling.

In the next step, the synthesis of the tripeptides was not straightforward and gave only very low yields (<30%) during the initial studies. One of the main problems was found during the *N*<sup>α</sup>-deprotection. Compared to an almost clean deprotection with the building block **3.15** itself, many more by-products were formed when subjecting one of the dipeptides to 20% TFA in DCM. To circumvent by-product formation, the deprotection was carried out at 0 °C, which resulted in prolonged reaction times, typically up to 4 hours. By this modification the by-product formation was almost completely suppressed and clean reactions were obtained.

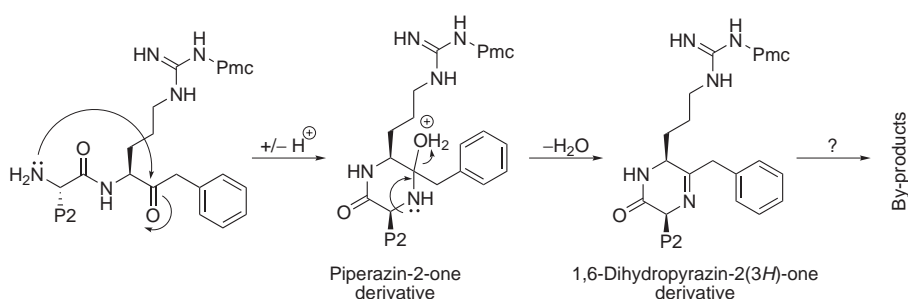
Having solved the problems in the deprotection step, new problems arrived with the second peptide coupling. Once again cooling to 0 °C solved some of the problems, but it was also found to be important at which point DIPEA was added. Significantly lower yields of the corresponding tripeptide was obtained if DIPEA was added to form the free amine of the dipeptide, before addition of the activated amino acid. This is quite reasonable because a free amine in the presence of an electrophilic group (the benzyl ketone) is subject to severe by-product formation. In fact, the free amine of the dipeptide is lined up for a cyclization giving a 6-membered ring, a piperazine-2-one derivative (see Scheme 3.2.3).



**Table 3.2.2:** *Synthesis of protected tripeptides (P3-P1).*

| entry | P4  | P3 <sup>a</sup> | P2 <sup>a</sup> | product     | yield (%) <sup>b</sup> | purity (%) <sup>c</sup> |
|-------|-----|-----------------|-----------------|-------------|------------------------|-------------------------|
| 1     | Boc | Tyr(Bn)         | Thr(Bn)         | <b>3.19</b> | 40–69                  | >69.3                   |
| 2     | Boc | Tyr(Bn)         | Leu             | <b>3.20</b> | 39–41                  | >79.2                   |
| 3     | Boc | Phe             | Leu             | <b>3.21</b> | 29–31                  | >85.2                   |
| 4     | Boc | Phe             | Phe             | <b>3.22</b> | 54                     | >88.5                   |
| 5     | Boc | D-Phe           | Phe             | <b>3.23</b> | 27                     | >79.6                   |
| 6     | Cbz | Phe             | Phe             | <b>3.24</b> | 32                     | 69.3                    |
| 7     | Cbz | D-Phe           | Phe             | <b>3.25</b> | 27                     | 80.6                    |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain. <sup>b</sup>: Purified yield. <sup>c</sup>: The lowest determined purity based on UPLC analysis (TFA).



**Scheme 3.2.3:** *The free amine of the dipeptide is lined up for a cyclization forming initially a piperazine-2-one derivative, which could lead to further by-product formation. However, the shown derivatives here have not been observed by LC-MS, but many other uncharacterized by-products have.*

This derivative is believed to eliminate water forming an enamine and then react further forming many by-products. However, the shown derivatives were not observed by

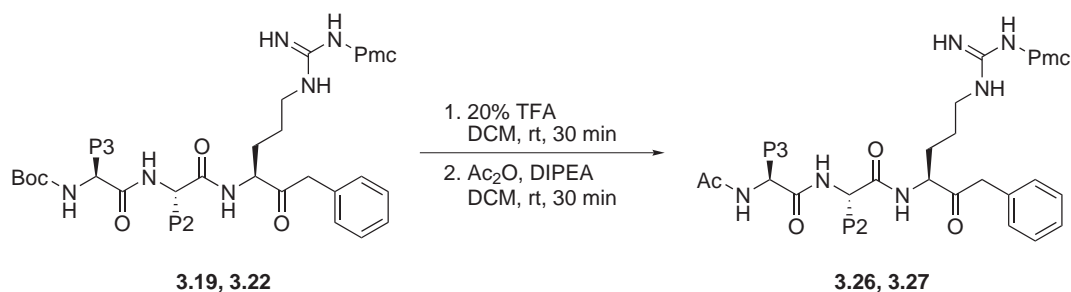
LC-MS, but many other by-products were formed most likely via these species. Nevertheless, addition of DIPEA after mixing the deprotected dipeptide and the activated *N*-protected amino acid usually solved the problem and reasonable purified yields of the tripeptides **3.19–3.25** were obtained (see Table 3.2.2). Some variation in the obtained yields was still observed, which indicates some lack of reproducibility. The tripeptides were generally also less pure than the dipeptides, even after flash chromatography (c.f. Tables 3.2.1 and 3.2.2).

Coupling using activated amino acids directly, such as Boc-Tyr(Bn)-OSu for the synthesis of Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**) was investigated, but no successful results were achieved with the dipeptide **3.16**. Only traces of **3.19** were formed based on LC-MS analysis along with many uncharacterized by-products. Using a preactivated amino acid was therefore not successful. Despite the problems with the synthesis of the tripeptides, the method still seems to be the best when using solution-phase synthesis.

### 3.2.3.2 Functionalization of the N-terminal

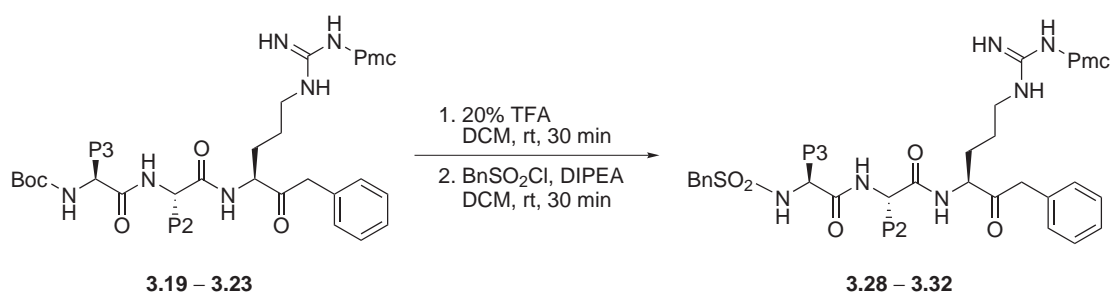
The acetyl *N*-terminal was easily introduced by chemoselective deprotonation of the *N*-terminal with 20% TFA in DCM followed by reaction with Ac<sub>2</sub>O in the presence of DIPEA. Upon purification, the *N*-acetylated tripeptides **2.26** and **3.27** were obtained in good yield (67–70%) (see Table 3.2.3). Only these two peptide sequences were selected because they represent two rather different rankings according to the FVIIa specificity profile previously described.

The *N*-sulfonylation (BnSO<sub>2</sub>) is known from the literature used for capping of amino groups,<sup>430,431</sup> as well as *N*-terminals for serine protease inhibitors.<sup>130,165,166</sup> Introduction of the *N*-BnSO<sub>2</sub>-terminal proceeded, after deprotection of the *N*<sup>α</sup>-protection, by reaction with BnSO<sub>2</sub>Cl in the presence of a DIPEA (see Table 3.2.4) according to a literature procedure.<sup>431</sup>

**Table 3.2.3:** *N*-Acetylation of tripeptides.

| entry | P4 | P3 <sup>a</sup> | P2 <sup>a</sup> | product     | yield (%) <sup>b</sup> | purity (%) <sup>c</sup> |
|-------|----|-----------------|-----------------|-------------|------------------------|-------------------------|
| 1     | Ac | Tyr(Bn)         | Thr(Bn)         | <b>3.26</b> | 67–70                  | >92.1                   |
| 2     | Ac | Phe             | Phe             | <b>3.27</b> | 70                     | >89.6                   |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain. <sup>b</sup>: Purified yield. <sup>c</sup>: The lowest determined purity based on UPLC analysis (TFA).

**Table 3.2.4:** *N*-Sulfonylation of tripeptides.

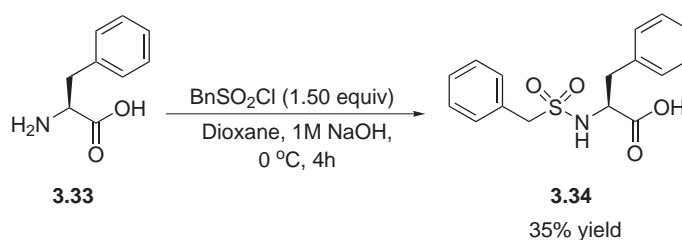
| entry | P4                | P3 <sup>a</sup> | P2 <sup>a</sup> | product     | conv. (%) <sup>b</sup> | purity (%) <sup>c</sup> |
|-------|-------------------|-----------------|-----------------|-------------|------------------------|-------------------------|
| 1     | BnSO <sub>2</sub> | Tyr(Bn)         | Thr(Bn)         | <b>3.28</b> | 35                     | >77.6                   |
| 2     | BnSO <sub>2</sub> | Tyr(Bn)         | Leu             | <b>3.29</b> | 79                     | >59.9                   |
| 3     | BnSO <sub>2</sub> | Phe             | Leu             | <b>3.30</b> | >95                    | >50.8                   |
| 4     | BnSO <sub>2</sub> | Phe             | Phe             | <b>3.31</b> | 80                     | >63.5                   |
| 5     | BnSO <sub>2</sub> | D-Phe           | Phe             | <b>3.32</b> | 67                     | >39.1                   |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain. <sup>a</sup>: Determined by LC-MS. <sup>c</sup>: The lowest determined purity based on UPLC analysis (TFA).

Unfortunately, the introduction of the *N*-BnSO<sub>2</sub>-terminal was not as easy as expected. Initially, the reaction was performed using 2.50 equiv, but that led to approximately 20% of the corresponding di(BnSO<sub>2</sub>)-product. Different equivs ranging from 1.10–2.50 were tested, and 1.10 equiv was found to be the best affording a maximized conversion and a minimized by-product formation. However, the reaction was rather unpredictable and sometimes full conversion was achieved within 30 minutes and in other cases full conversion was never reached. Besides of different equiv BnSO<sub>2</sub>Cl, different temperatures (0 °C to reflux), different reaction times and different bases (DIPEA, Et<sub>3</sub>N and DMAP) were tested, but in all cases the reaction remained tricky. Inspired by the previous results, DIPEA was not added to the deprotected tripeptide until BnSO<sub>2</sub>Cl was added.

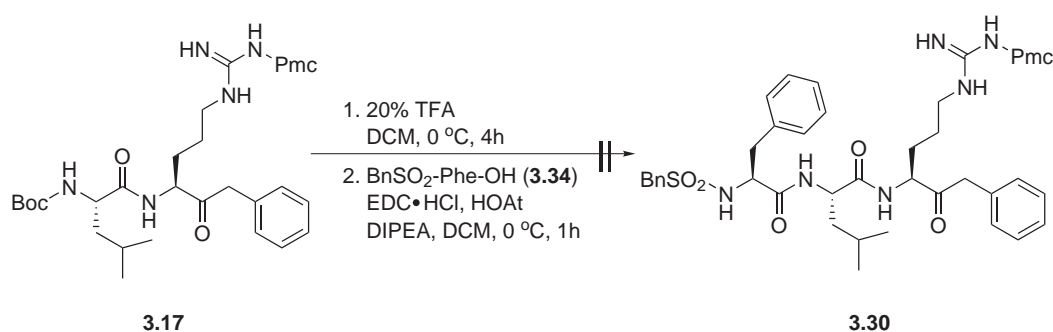
As well as unconverted starting material and product, the crude products also contained many minor uncharacterized products as well as the di(BnSO<sub>2</sub>)-product. The rather impure crude products were subjected to flash chromatography, but a successful procedure was not found. Therefore, several of the *N*-sulfonylated peptides were used without further purification and the impurities were removed upon preparative HPLC of the final compounds.

In efforts to optimize the *N*-sulfonylation, another strategy was tested. In the literature, this *N*-BnSO<sub>2</sub> moiety has also been introduced directly along with the last amino acid, serving as a *N*-protection during the last peptide coupling.<sup>165,166</sup> Because the introduction of the *N*-BnSO<sub>2</sub>-group seems to be rather sluggish with these substrates, it would be more efficient to introduce it with the P3-amino acid. Hereby, the low-yielding reaction step is performed on a cheap starting material in stead of on an expensive and synthetically time-consuming dipeptide.



**Scheme 3.2.4:** *N*-Terminal modification of *H*-Phe-OH (**3.33**) into the corresponding BnSO<sub>2</sub>-amino acid **3.34** in moderate yield according to a literature procedure.<sup>432</sup>

To test this strategy, H-Phe-OH (**3.33**) was converted into the corresponding *N*-sulfonylated amino acid **3.34** in moderate yield according to a literature procedure<sup>432</sup> (see Scheme 3.2.4).



**Scheme 3.2.5:** Coupling the dipeptide **3.17** with the *N*-sulfonylated amino acid **3.34** did surprisingly not give the desired product *BnSO<sub>2</sub>*-Phe-Leu-Arg(*Pmc*)-*bk* (**3.30**).

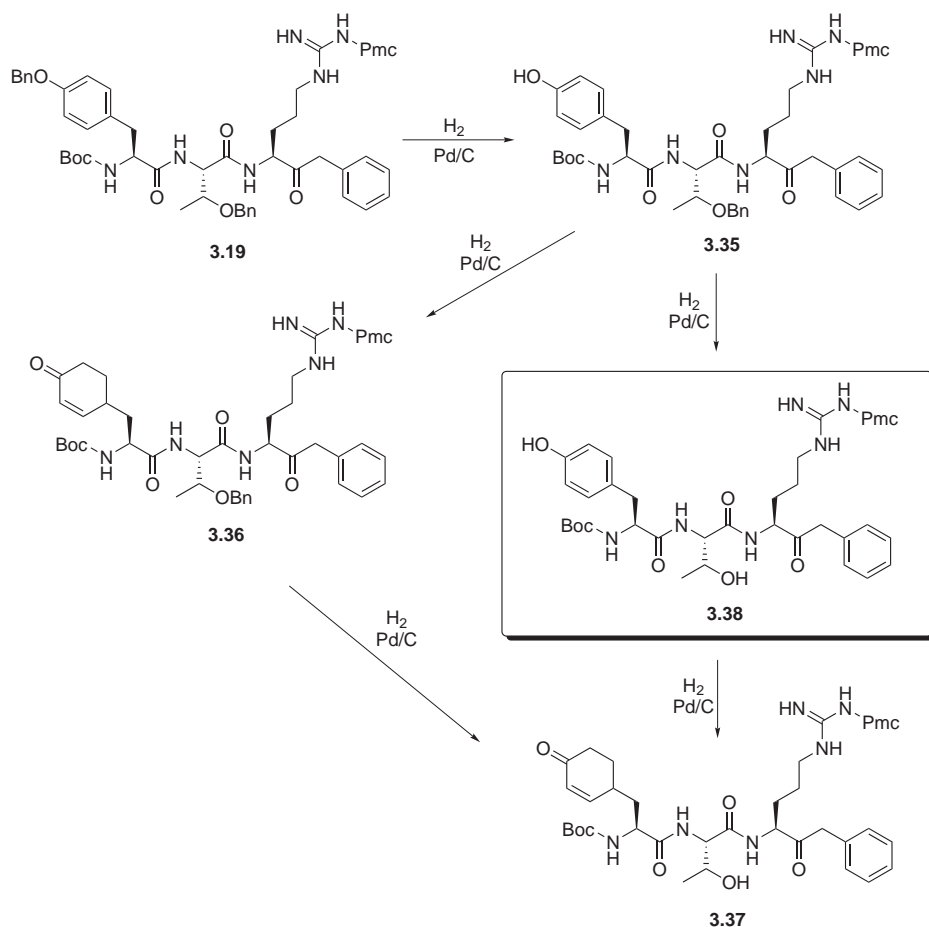
With the new P4/P3-amino acid **3.34** in hand, its usability was tested for the synthesis of *BnSO<sub>2</sub>*-Phe-Leu-Arg(*Pmc*)-*bk* (**3.30**) under the previously optimized conditions for synthesis of tripeptides (see Scheme 3.2.5).

The coupling of the dipeptide **3.17** with **3.34** did surprisingly not give the desired product **3.30** despite that this approach has been reported by Parlow and co-workers.<sup>165,166</sup> Instead of forming any product, many uncharacterized compounds were formed. No further optimization was pursued.

### 3.2.3.3 *O*-Debenzylation of tyrosine and threonine

*O*-Debenzylation of Boc-Tyr(Bn)-Thr(Bn)-Arg(*Pmc*)-*bk* was initially investigated using catalytic hydrogenolysis over Pd/C (see Scheme 3.2.6 and Table 3.2.5).

The desired product **3.38** was obtained in 14% based on LC-MS when the hydrogenolysis was performed with 3 bar H<sub>2</sub> at room temperature overnight in EtOH (see entry 1). The two main components in the reaction mixture were unreacted starting material **3.19** and the mono *O*-debenzylated product **3.35** (see Scheme 3.2.6). This experiment clearly indicated that *O*-debenzylation of tyrosine was much faster than *O*-debenzylation of threonine.



**Scheme 3.2.6:** Catalytic hydrogenolysis over Pd/C of Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**) was investigated. Deprotection of threonine was much slower than that of tyrosine and two reduced by-products (**3.36** and **3.37**) were sometimes also observed.

By increasing the pressure of H<sub>2</sub> 80 bar indeed forced the reaction toward the products, but unfortunately, the main product (84%) was just the mono-*O*-debenzylated one **3.35** and only 16% of the desired product **3.38** was obtained (entry 2). Changing the solvent to MeOH gave no product and only the mono-*O*-debenzylated compound was found in the crude product (entry 3). To force the reaction towards the product **3.38**, a series of experiments in MeOH at elevated temperature was carried out (entries 4–6). Up to 39% **3.38** was observed, but large amounts of the two reduced compounds **3.36** and **3.37** were also obtained (up to 45%). These two compounds are believed to be the mono- and the di-*O*-debenzylated peptides, respectively, each containing a reduced tyrosine residue.



periments at room temperature with reaction times of at least 24 hours were performed (entries 10–12). Gratifyingly, 80 bar at room temperature with a reaction time of 24 hours gave 87% of the desired product Boc-Tyr-Thr-Arg(Pmc)-bk (**3.38**) and only minor amounts of the reduced tyrosine compounds **3.37** (7%) and **3.35** (6%) were formed. Prolonged reaction time did not increase the amount of product but instead led to an increased amount of the di-*O*-debenzylated reduced tyrosine **3.37**. Last but not least, gently heating to 30 or 35 °C, respectively was tested, but that was not beneficial.

The optimization of the *O*-debenzylation was also tested on the  $N^\alpha/N^\omega$ -deprotected tripeptide H-Tyr(Bn)-Thr(Bn)-Arg-bk. Once again it was found, that deprotection of tyrosine occurred much faster than that of threonine. Steric hindrance from the Boc- or Pmc-groups was therefore not seen to be the reason for the difficulties of unprotecting the threonine residue. It is more reasonable that the difference in reaction kinetics is a result of the corresponding stability of the anions (phenolate ions are stabilized by resonance).

Usage of the Pearlman's catalyst ( $\text{Pd}(\text{OH})_2/\text{C}$ ) in combination with Pd/C has been described in the literature as a more active catalytic system for *O*-debenzylation.<sup>355</sup> Therefore, the hydrogenolysis of **3.19** was tested with different loadings (10–25 wt%) of Pd/C: $\text{Pd}(\text{OH})_2/\text{C}$  (1:1) at different pressures (3–80 bar). Unfortunately, it turned out that this catalytic system was too active and many by-products were formed. Less  $\text{Pd}(\text{OH})_2/\text{C}$  was also tested with Pd/C, but was not beneficial. Reduction of the benzyl ketone was probably one of the new by-products observed with the Pearlman's catalyst (based on LC-MS). Apparently, the more active catalyst is capable of reducing the ketone functionality. Addition of 10% AcOH and decrease of  $\text{H}_2$  pressure to 3 bar did not give any successful results.

Lewis acid-promoted *O*-debenzylation was also tested with the  $\text{SnCl}_4$  and  $\text{TiCl}_4$ , respectively. Once again it was observed that threonine was much more difficult to deprotect than tyrosine. Even though an excess of the Lewis acid was used at reflux, no clean deprotection was observed and this strategy was terminated.

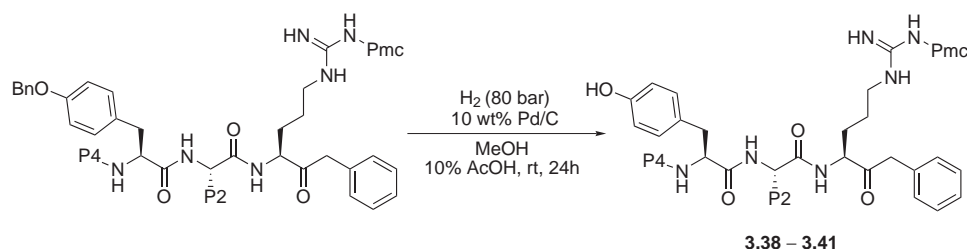
Based on the optimization results, the catalytical hydrogenolysis at 80 bar  $\text{H}_2$  at room temperature for 24 hours gave the best result (see Table 3.2.5, entry 11). Therefore the synthesized tripeptides were subjected to these conditions for *O*-debenzylation of those peptides containing tyrosine and threonine residues (see Table 3.2.6).

Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**) and Ac-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.26**) were both successfully *O*-debenzylated (conv. >90%) giving only minor amounts of the corresponding mono-reduced species (5–10%) (entries 1–2). The deprotected peptides were used without further purification. Subjecting the *N*-sulfonylated peptides **3.28** and



**3.29** to the very same reaction conditions unfortunately did not afford any traces of the desired products **3.40** or **3.41**, respectively. The reaction time was increased to several days, but only a mixture of the starting material and the mono-*O*-debenzylated compounds were observed based on LC-MS analysis. Apparently, the benzylsulfonyl *N*-terminal decreases the reactivity or is causing catalyst poisoning, maybe due to coordination to sulfur. Further optimization was not pursued, and the synthesis of BnSO<sub>2</sub>-Tyr-Thr-Arg-bk (**3.40**) and BnSO<sub>2</sub>-Tyr-Leu-Arg-bk (**3.41**) did not succeed.

**Table 3.2.6:** Hydrogenolysis for *O*-debenzylation of tyrosine and threonine.



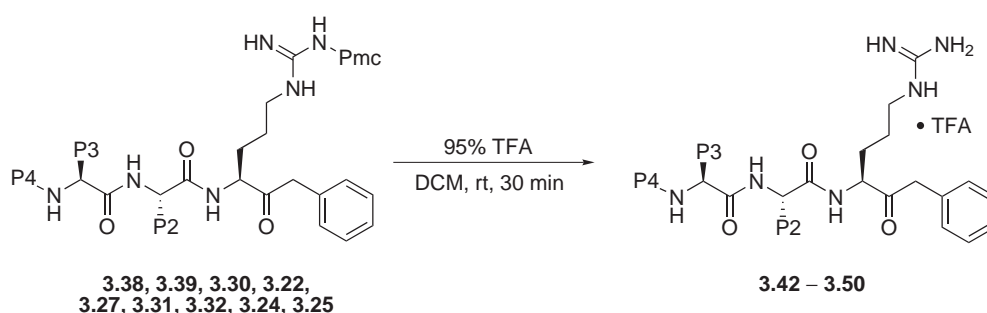
| entry | P4 <sup>a</sup>   | P3 <sup>a</sup> | P2 <sup>a</sup> | product     | conv. (%) <sup>b</sup> | purity (%) <sup>c</sup> |
|-------|-------------------|-----------------|-----------------|-------------|------------------------|-------------------------|
| 1     | Boc               | Tyr             | Thr             | <b>3.38</b> | 90 (5)                 | >80.1                   |
| 2     | Ac                | Tyr             | Thr             | <b>3.39</b> | 95 <sup>d</sup> (10)   | –                       |
| 3     | BnSO <sub>2</sub> | Tyr             | Thr             | <b>3.40</b> | – <sup>e</sup>         | –                       |
| 4     | BnSO <sub>2</sub> | Tyr             | Leu             | <b>3.41</b> | – <sup>e</sup>         | –                       |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain. <sup>b</sup>: Conversion based on LC-MS. Amount of reduced by-product stated in the brackets (%). <sup>c</sup>: The lowest determined purity based on UPLC analysis (TFA). <sup>d</sup>: A reaction time of 2.5 days was required for full conversion. <sup>e</sup>: No product was formed after 2.5 days. Mainly the mono-*O*-debenzylation was observed on LC-MS.

### 3.2.3.4 Deprotection, purification and lyophilization

Those peptides successfully synthesized were carried on to the final Boc/Pmc-deprotection which was easily performed by treatment with 95% TFA in DCM. The crude products were purified by preparative HPLC using an appropriate gradient of H<sub>2</sub>O→MeCN. Upon lyophilization of the pure fractions and nitrogen quantification by HPLC-CLND, nine peptidyl benzyl ketones **3.42–3.50** were obtained in good (see Table 3.2.7).

Although the yields are modest (some of them are actually based on three steps), the purity was prioritized when selecting the fractions from the preparative HPLC purification. In all cases the purified peptide was lyophilized yielding the corresponding TFA-salt

**Table 3.2.7:** Final deprotection and purification by preparative HPLC.

| entry | peptide                             | product     | yield (%) <sup>b</sup> | purity (%) <sup>c</sup> |
|-------|-------------------------------------|-------------|------------------------|-------------------------|
| 1     | H-Tyr-Thr-Arg-bk                    | <b>3.42</b> | 35 <sup>d</sup>        | >93.0                   |
| 2     | Ac-Tyr-Thr-Arg-bk                   | <b>3.43</b> | 14 <sup>d</sup>        | >87.9                   |
| 3     | BnSO <sub>2</sub> -Phe-Leu-Arg-bk   | <b>3.44</b> | 30                     | >95.3                   |
| 4     | H-Phe-Phe-Arg-bk                    | <b>3.45</b> | 49                     | >95.4                   |
| 5     | Ac-Phe-Phe-Arg-bk                   | <b>3.46</b> | 57                     | >95.9                   |
| 6     | BnSO <sub>2</sub> -Phe-Phe-Arg-bk   | <b>3.47</b> | 24 <sup>d</sup>        | >94.5                   |
| 7     | BnSO <sub>2</sub> -D-Phe-Phe-Arg-bk | <b>3.48</b> | 29 <sup>d</sup>        | >85.0 <sup>e</sup>      |
| 8     | Cbz-Phe-Phe-Arg-bk                  | <b>3.49</b> | 21                     | >74.3 <sup>e</sup>      |
| 9     | Cbz-D-Phe-Phe-Arg-bk                | <b>3.50</b> | 22                     | 83.4 <sup>e</sup>       |

<sup>a</sup>: Notation corresponds to the respective amino acid side-chain. <sup>b</sup>: Yield (2 steps) determined by HPLC-CLND nitrogen quantification of purified peptide. <sup>c</sup>: The lowest determined purity based on UPLC analysis (TFA, NH<sub>4</sub>HCO<sub>3</sub> and/or Na<sub>2</sub>SO<sub>4</sub>). <sup>d</sup>: Yield based on three steps, because N-terminal modification and/or deprotection step was used directly without purification. <sup>e</sup>: UPLC (TFA) gave a purity of higher than 95%.

as a white solid compound.

### 3.2.4 Biological activity and SAR of the FVIIa inhibitors

The synthesized peptides were all subjected to three competitive binding assays for screening of inhibitory activity against FVIIa, thrombin (thr) and FXa, respectively (see Table 3.2.8). Solubility of the peptides in the assay buffer (50 mM HEPES, 100 mM NaCl, 6.6 mM CaCl<sub>2</sub>, 0.01% Tween80) varied a lot. The tyrosine- and threonine-containing peptides **3.42** and **3.43** were highly soluble in the buffer, whereas the *N*-sulfonylated peptides **3.44**, **3.47** and **3.48** and in particular the Cbz-modified peptides **3.49** and **3.50** were much less soluble. The highest concentration tested in the assay therefore varied accordingly, but in general concentrations of more than 1400 μM were not considered.

**Table 3.2.8:** *Inhibition of blood coagulation serine proteases.*

| entry | peptide     | IC <sub>50</sub> (μM) <sup>a</sup> |       |       | K <sub>i</sub> (μM) | FVIIa/thr <sup>b</sup> | FVIIa/FXa <sup>b</sup> |
|-------|-------------|------------------------------------|-------|-------|---------------------|------------------------|------------------------|
|       |             | FVIIa                              | thr   | FXa   |                     |                        |                        |
| 1     | <b>3.42</b> | >1340                              | >1340 | >1340 | –                   | –                      | –                      |
| 2     | <b>3.43</b> | >1380                              | >1380 | 1078  | –                   | –                      | >0.78                  |
| 3     | <b>3.44</b> | >580                               | >580  | >580  | –                   | –                      | –                      |
| 4     | <b>3.45</b> | >1480                              | >1480 | >1480 | –                   | –                      | –                      |
| 5     | <b>3.46</b> | >400                               | >400  | >400  | –                   | –                      | –                      |
| 6     | <b>3.47</b> | 26                                 | >590  | >590  | 43                  | >23                    | >23                    |
| 7     | <b>3.48</b> | 640                                | >640  | >640  | –                   | >1                     | >1                     |
| 8     | <b>3.49</b> | 109                                | >650  | 508   | 89                  | >6                     | >5                     |
| 9     | <b>3.50</b> | 16                                 | 560   | 440   | 8                   | 35                     | 28                     |

<sup>a</sup>: Estimated IC<sub>50</sub>-value determined by a competitive binding assay against FVIIa, thrombin or FXa, respectively. Concentration of inhibitor was determined by HPLC-CLND. <sup>b</sup>: Selectivity over thrombin and FXa, respectively, calculated using the IC<sub>50</sub> values.

The most potent FVIIa inhibitor was found to be Cbz-D-Phe-Phe-Arg-bk (**3.50**) and has an IC<sub>50</sub> = 16 μM and K<sub>i</sub> = 8 μM (entry 9). The inhibitor has a 35- and 28-fold selectivity over thrombin and FXa, respectively. Two less potent inhibitors were found to be BnSO<sub>2</sub>-Phe-Phe-Arg-bk (**3.47**) with K<sub>i</sub> = 43 μM and Cbz-Phe-Phe-Arg-bk (**3.49**) with K<sub>i</sub> = 89 μM (entries 6 and 8). Inhibitor **3.47** possesses at least a 23-fold selectivity against both thrombin and FXa, whereas **3.49** is almost equal potent against all three blood coagulation enzymes. Cbz-D-Phe-Phe-Arg-bk (**3.50**) therefore represents the most potent and selective FVIIa inhibitor of them all.

Based on the biological results, a bulky and aromatic *N*-terminal is crucial for activity. This P4-moiety is believed to probe the S4 and/or the subsite of the S1 binding pocket.<sup>130,165,166</sup> *N*-Sulfonylation has been reported previously,<sup>115,116,130,165,166</sup> but Cbz-modified peptides have only briefly been described in the literature in connection with thrombin inhibitors (c.f. **1.12**, Figure 1.1.16). Although it was concluded from the FVIIa-TF specificity profile, that the S4 binding site is undefined,<sup>168</sup> these results show that probing of S4 binding pocket is highly important with this class of inhibitors.

The influence of a D-amino acid at P3 is still not fully understood. Cbz-D-Phe-Phe-Arg-bk (**3.50**) was slightly more potent than the corresponding L-diastereomer **3.49**. The reverse results were observed with BnSO<sub>2</sub>-Phe-Phe-Arg-bk (**3.47**) and BnSO<sub>2</sub>-D-Phe-Phe-Arg-bk (**3.48**); the former was a potent FVIIa inhibitor with IC<sub>50</sub> = 26 μM (K<sub>i</sub> = 43 μM), but the latter was approximately 25-fold less potent (IC<sub>50</sub> = 640 μM). The effect of introducing a D-amino acid at P3 is not uniform among the inhibitors. However, these results clearly show that D-amino acids are not only used for increasing the *in vivo* stability, but also for altering the potency of the inhibitor.

Not surprisingly, the amino acid at P2 has an important role. If phenylalanine is compared to leucine, the latter should be a more active amino acid according to the FVIIa-TF specificity profile.<sup>168</sup> However, that was not observed experimentally in this study. Comparing BnSO<sub>2</sub>-Phe-Leu-Arg-bk (**3.44**) and BnSO<sub>2</sub>-Phe-Phe-Arg-bk (**3.47**) shows that **3.47** is more than 20 times more potent against FVIIa than **3.44**.

Eventhough H-Tyr-Thr-Arg-bk (**3.42**) and Ac-Tyr-Thr-Arg-bk (**3.43**) have the supposedly most potent S3-S1 scaffolds, these compounds were not significantly active against any of the tested serine proteases. Threonine is most likely forming a hydrogen bond with Asp60 through a water molecule, which is a unique feature of the S2 binding pocket of FVIIa. Despite such a favored interaction with the enzyme, the contribution of the P4 group is apparently of much more importance. Presumably, this group forms hydrogen bonds with Lys192 and/or Gly219 as well as hydrophobic interactions within the S4 binding pocket. These bindings are apparently essential for achieving good potency with this class of FVIIa inhibitors.

These results moreover indicate that a specificity profile cannot directly be translated into synthetic compounds with similar biological activity. The main reason for this is undoubtedly due to interdependence resulting in conformational changes of the enzyme upon binding of a certain substrate. These changes alter the specificity, and therefore it is rather unpredictable.

Comparing Cbz-D-Phe-Phe-Arg-bk (**3.50**) with some of the reported FVIIa peptidyl inhibitors, this inhibitor is less potent compared to the most potent inhibitors reported. For example, the α-ketothiazole **1.23** presented in Section 1.1.3.3 has a K<sub>i</sub> = 0.20 μM against FVIIa, and thus is 40 times more potent than **3.50**.<sup>165,166</sup> It also got a superior selectivity against thrombin (500-fold), but no selectivity against FXa. One possible explanation for the increased potency of **1.23** is that the α-ketothiazole is very electron-withdrawing giving a electron-deficient and reactive carbonyl group. The benzyl ketone is less electrophile, but still having the aromatic moiety, which in fact has been found to be the most important part of the P1' part of the molecule. In this regard, functionalization of the benzyl ketone would be a valuable extension of the SAR of these inhibitors.

Selection of the most appropriate inhibitor for a liquid formulation of FVIIa is based on many parameters. A potency in the low micromolar is likely a sufficient inhibitor to gain some stabilization of FVIIa in a liquid formulation. Naturally, it is decided to minimize the amount of inhibitor in the formulation, but on the other hand, a highly potent inhibitor would not be desirable. FVIIa binds to the surface of the thrombin-activated platelets with low affinity (c.f. Figure 1.1.5). If a highly potent inhibitor was used, the pharmacological response would probably be impaired, which is not desirable. Selectivity against other blood coagulation serine proteases is another parameter to consider. For the usage as a stabilization agent, this issue is not as important than if the inhibitor should itself be used in pharmacological doses. When co-injecting the inhibitor with FVIIa, the resulting dilution is probably high enough to prevent the inhibitor to inhibit any of the other coagulation factors substantially. In this regard, a high *in vivo* clearance would be desirable.

Evaluating Cbz-D-Phe-Phe-Arg-bk (**3.50**) as a potential stabilization agent, it has a practicable potency and selectivity. However, the solubility in aqueous media is low and that is probably the biggest concern. Introduction of polar functionalities, e.g. at the P1' position or as a part of the S4-S1 scaffold would contribute to a better solubility. This was observed for the Tyr-Thr-containing peptides, which had a much better solubility. The development of FVIIa inhibitors for liquid formulations of NovoSeven<sup>®</sup> is not complete yet and further optimization would be required before the desired goal has been achieved. Besides of the medicinal chemistry part of the project, development of new formulations of FVIIa should be investigated, and especially the long-term stability of the enzyme is of interest. Solutions of FVIIa (e.g. in the assay buffer) are stable for at least a week at room temperature, because the proteolytic activity is significantly reduced in the absence of TF. Evaluation of the long-term stability should therefore be done over the course of several months. In addition, the activity of FVIIa should be determined in a clotting assay measuring the ability of the enzyme to initiate the blood coagulation cascade. These future applicabilities were however beyond the scope of this thesis.

### 3.3 Conclusion

The arginyl benzyl ketone building block **3.15** was synthesized from Boc-Arg(Pmc)-OH (**3.13**) via the Weinreb amide **3.14** in good yield without racemization. The P3-P1 sequence of the target peptides was selected from a previously reported FVIIa-TF specificity profile, giving that P1 should always be arginine and P3-P2 should be combinations of tyrosine, threonine, phenylalanine and leucine. Different *N*-terminals (P4; H-, Ac-, BnSO<sub>2</sub>- and Cbz-) were also examined. Thus, a small library of peptidyl benzyl ketones was prepared by a Boc/Bn solution-phase peptide synthetic approach. Removal of the *N*<sup>α</sup>-deprotection was accomplished selectively with 20% TFA in DCM followed by an EDC/HOAt-mediated peptide coupling. The first coupling proceeded smoothly, but the second was challenging because of severe by-product formation. Through optimization, this problem was solved by running the deprotection and the peptide coupling at 0 °C. Furthermore, the order of addition was found to be crucial, in particular it was important that DIPEA was added after mixing the deprotected amine and the activated amino acid. A *N*-sulfonylation was introduced with BnSO<sub>2</sub>Cl, but the reaction was found to be very sluggish and not reproducible. Screening of different reaction conditions did not lead to optimization of the reaction. Tyrosine and threonine were *O*-debenzylated using catalytic hydrogenolysis at 80 bar H<sub>2</sub> over 10wt% Pd/C in 10% AcOH/MeOH. This gave full conversion of the desired Boc- or *N*-acetylated peptides **3.38** and **3.39**, with a minimal reduction of the tyrosine residue. The deprotection was found to be rather difficult and especially the *O*-debenzylation of threonine was slow. Unfortunately these optimized conditions were ineffective for *O*-deprotection of *N*-sulfonylated peptides, such as **3.40** and **3.41**. This limitation restricts the number of tyrosine- and threonine-containing compounds when using the solution-phase synthetic approach. Thus, nine peptidyl benzyl ketones were synthesized (**3.42–3.50**) and their biological activity was evaluated in competitive binding assays for FVIIa, thrombin and FXa. The most potent FVIIa inhibitor was identified as Cbz-D-Phe-Phe-Arg-bk (**3.50**) with a  $K_i = 8 \mu\text{M}$  ( $\text{IC}_{50} = 16 \mu\text{M}$ ) and with a 35- and 28-fold selectivity against thrombin and FXa, respectively. A bulky aromatic *N*-terminal was crucial for activity. Cbz was found to be superior compared to BnSO<sub>2</sub>. Eventhough the specificity profile showed that Tyr-Thr-Arg should be the most potent sequence, the results here show that D-Phe-Phe-Arg in fact was the most potent sequence of the synthesized compounds. Usability of Cbz-D-Phe-Phe-Arg-bk (**3.50**) as a stabilizing agent for liquid formulations of FVIIa still remains unexplored, but based on the potency and selectivity this inhibitor seems to be a promising candidate. However, **3.50** is rather insoluble in aqueous media which is a potential problem for the usage in a drug formulation. Further optimization of the synthetic strategy would be necessary to prepare Tyr-Thr-Arg-containing peptides with the appropriate *N*-terminals as well as a thorough SAR study of the P1' position.



An entirely solid-supported synthesis using mostly standardized Fmoc-strategy could potentially be achieved with the DHUA-linker, which has been used for the synthesis of a peptide aldehyde (c.f. Scheme 3.1.4).<sup>406</sup> It is believed that the benzyl ketone building block **3.15** could be anchored to the linker mediated by TMSOTf giving the acetal linker **3.51** (see Scheme 3.4.1). This acetal furthermore serves as a protecting group preventing side-reactions with the electrophilic carbonyl during peptide synthesis. The linker can be anchored to the solid-support by traditional coupling conditions (BOP, HOBt, DIPEA) affording **3.52**. These two steps are critical and important for utilization of this method.

Then a selective  $N^\alpha$ -deprotection has to be developed. If the conditions are too acidic, the Pmc group will also be removed as well as cleaving the acetal. Mild and non-standardized removal conditions may be effective. The free amine **3.53** is then thought to be subjected to two consecutive peptide coupling reactions using a standardized Fmoc/*t*Bu approach (e.g. DIC/HOBt, DIPEA)<sup>328</sup> leading to a tripeptide, for example **3.54**, which is thought to be cleaved from the resin and deprotected with 95% TFA in H<sub>2</sub>O leading to the desired inhibitor **3.55**. One major advantage by using this solid-phase approach would be the choice of tyrosine and threonine *O*-protection. When using the Fmoc-strategy, the hydroxy group is generally protected as a *tert*-butyl ether which is readily removed by treatment with acid. This synthetic approach would simplify the preparation of Tyr-Thr-Arg-containing peptides, which is of great interest due to their potential biological activity and improved solubility in aqueous media.



## 3.5 Experimental

### General procedures - chemistry

Chemicals and reagents were all commercially available and used without further purification. Solvents were of HPLC quality and used without further purification. Evaporation *in vacuo* was performed on a rotary evaporator at approx. 40 °C down to approx. 20 mbar. TLC was performed using silica gel 60 F<sub>254</sub> aluminum sheets. The plates were visualized in UV light (254 nm) and by staining with 5% MoO<sub>3</sub>/H<sub>3</sub>PO<sub>4</sub> in EtOH and subsequently heated with a heating gun. Flash chromatography was performed on a Biotage<sup>®</sup> FlashMaster purification system. The eluent ratios given are vol%. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were recorded on a Bruker Avance DRX 400 instrument using deuterated solvents as internal references. Chemical shifts ( $\delta$ ) are given in ppm and coupling constants ( $J$ ) in Hertz. HRMS was performed on an Agilent Technologies 1200 Series LC/MSD-TOF instrument using a C18 column (5→95% MeCN in H<sub>2</sub>O, 15 minutes). The found mass ( $m/z$ ) is stated along with the apparent fragment, which varies less than 5 ppm compared to the calculated exact mass. MS was determined on a Perkin Elmer 200 Series PE Sciex 3000 LC/MS/MS Turbo Ionspray system (5→90% MeCN in H<sub>2</sub>O, 10 minutes). Analytical UPLC was performed on a Waters Acquity Ultra Performance LC using three different systems; TFA: 5→95% MeCN (or variations hereof) in H<sub>2</sub>O (16 minutes) containing 0.05% TFA, NH<sub>4</sub>HCO<sub>3</sub>: 10→40% MeCN (or variations hereof) in H<sub>2</sub>O (16 minutes) containing 0.25M NH<sub>4</sub>HCO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>: 40→70% MeCN (or variations hereof) in H<sub>2</sub>O (13 minutes) containing 0.2M Na<sub>2</sub>SO<sub>4</sub> and 0.04M H<sub>3</sub>PO<sub>4</sub> (pH 3.5). Melting points were measured on a Büchi 535 Melting Point Apparatus and given in degrees Celsius (°C), uncorrected. IR analysis was carried out on a Bruker Alpha FT-IR spectrometer using attenuated total reflection (ATR) sampling technique. Only the strongest or structurally most significant peaks are included in the experimental section, stated in cm<sup>-1</sup>. Chiral HPLC was run on Waters Separation Module e2695 with a Photodiode Array Detector 2998 at 214 nm and 254 nm wavelengths. A Chiralcel<sup>®</sup> OD-H (cellulose tris(3,5-dimethylphenylcarbamate) coated on 5  $\mu$ m silica-gel) from Chiral Technologies Europe was used with an isocratic eluent consisting of 80% hexanes and 20% *i*PrOH (1 mL/min, rt, 650 psi). The enantiomeric excess was calculated based on an average of the integral areas. Purification by preparative, reverse-phase HPLC was performed on a Waters DeltaPrep system using a Phenomenex C18 column (250×12.2Ø mm, 5 micron) with UV/Visible detection on a Waters 2480 Detector. Fractions were analyzed by LC-MS and analytical HPLC and all pure fractions were combined and evaporated *in vacuo* to remove most of the MeCN. The residue was transferred to one or more freeze-drying glasses, capped with a lid filter and frozen quickly by immersion in an acetone/dry ice bath (-78 °C). When complete frozen, the compound was lyophilized in a Christ Alpha 2-4 LSC Lyo Chamber (room temperature, 0.001 mbar) for one or two days depending on the batch size. Products were obtained as TFA-salts which were quantified by HPLC-CLND on an Agilent Tech-

nologies 1200 Series connected to an Agilent Interface 35900E / Antek HPLC-CLND.

**(2S)-5-(3-(2,2,5,7,8-Pentamethyl-3,4-dihydro-2H-chromen-6-ylsulfonyl)guanidino)-2-(tert-butoxycarbonyl)amino-N-methoxy-N-methylpentanamide (Boc-Arg(Pmc)-weinreb amide) (3.14)**

Boc-Arg(Pmc)-OH (**3.13**) (4.80 g, 8.88 mmol, 1.00 equiv) was suspended in DCM (60 mL), and subsequently dissolved by addition of Et<sub>3</sub>N (1.36 mL, 1.35 g, 13.32 mmol, 1.50 equiv). PyBOP (6.93 g, 13.32 mmol, 1.50 equiv) was added and after a few minutes followed by *N,O*-dimethylhydroxylamine hydrochloride (1.30 g, 13.32 mmol, 1.50 equiv) and Et<sub>3</sub>N (1.36 mL, 1.35 g, 13.32 mmol, 1.50 equiv). The clear, pale yellow solution was stirred at room temperature for 1 hour and then pH was adjusted to 5–6 by addition of Et<sub>3</sub>N. Stirring was continued for another 1 hour. The colorless reaction mixture was diluted with DCM (250 mL), washed successively with 1M HCl (2 × 100 mL), sat. aqueous NaHCO<sub>3</sub> (2 × 100 mL) and finally with brine (2 × 100 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. Purified by flash chromatography (50→90% EtOAc in heptane) afforded the Weinreb amide **3.14** as a white foam (4.81 g, 93%). *R*<sub>f</sub> = 0.31 (EtOAc). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.29 (broad s, 1H), 6.12 (s, 2H), 5.49 (d, *J* = 8.3 Hz, 1H), 4.68–4.64 (m, 1H), 3.74 (s, 3H), 3.44–3.32 (m, 1H), 3.20 (s, 3H), 3.17–3.11 (m, 1H), 2.63 (t, *J* = 6.7 Hz, 2H), 2.58 (d, *J* = 5.8 Hz, 6H), 2.11 (s, 3H), 1.82–1.79 (m, 2H), 1.72 (s, 3H), 1.68–1.48 (m, 3H), 1.42 (s, 9H), 1.31 (s, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 172.5, 156.3, 156.0, 153.5, 135.6, 134.9, 133.5, 124.0, 117.9, 80.3, 73.6, 61.6, 46.3, 41.0, 32.8, 32.1, 31.1, 28.3, 26.7, 24.7, 21.4, 18.4, 17.4, 12.1. HRMS (*m/z*) calcd. for C<sub>27</sub>H<sub>46</sub>N<sub>5</sub>O<sub>7</sub>S [M + H]<sup>+</sup>: 584.3113, found: 584.3141. HPLC purity (TFA): >97.2%. Mp 93–97 °C. IR (neat) *ν* 3436, 3340, 2974, 2934, 1707, 1650, 1620, 1545 (strong), 1449, 1367, 1246, 1163, 1105, 607 cm<sup>-1</sup>.

**(3S)-6-(3-(2,2,5,7,8-Pentamethyl-3,4-dihydro-2H-chromen-6-ylsulfonyl)guanidino)-3-(tert-butoxycarbonyl)amino-1-phenylhexan-2-one (Boc-Arg(Pmc)-bk) (3.15)**

Weinreb amide **3.14** (8.03 mmol, 1.00 equiv) was dissolved in dry THF (30 mL) under an atmosphere of N<sub>2</sub>. The colorless solution was cooled to 0 °C and 2.0M BnMgCl in THF (22.0 mL, 7.27 g, 48.19 mmol, 6.00 equiv) was added dropwise while stirring. Stirring at 0 °C was continued for 10 minutes and then the reaction mixture was slowly heated to room temperature over the course of 2 hours. The brownish reaction mixture was cooled to 0 °C again and 1M HCl (35 mL) was carefully added while stirring at such a rate that the temperature did not rise above 20 °C. The mixture was transferred to a separatory funnel with EtOAc (70 mL) and the aqueous layer was discarded. The organic layer was washed with 1M HCl (2 × 35 mL), sat. aqueous NaHCO<sub>3</sub> (2 × 35 mL) and brine (2 × 35 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness *in vacuo*. Purification by flash chromatography (10→70% EtOAc in heptane) afforded

the desired benzyl ketone as a white solid (3.54 g, 72%).  $R_f = 0.25$  (75% EtOAc in heptane).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.30–7.21 (m, 3H), 7.15–7.13 (m, 2H), 6.17 (s, 3H), 5.46 (d,  $J = 7.6$  Hz, 1H), 4.32 (m, 1H), 3.78–3.69 (m, 2H), 3.26–3.02 (m, 2H), 2.61 (d,  $J = 5.6$  Hz, 2H) 2.55 (d,  $J = 7.3$  Hz, 6H), 2.09 (s, 3H), 1.79–1.17 (m, 4H), 1.50 (broad s, 3H), 1.40 (s, 9H), 1.29 (s, 6H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  206.8, 156.1, 153.6, 135.5, 134.8, 133.2, 129.6, 128.7, 127.2, 124.1, 117.9, 80.2, 73.6, 46.4, 40.7, 32.7, 29.1, 28.3, 26.7, 25.0, 21.4, 18.5, 17.5, 12.1. HRMS ( $m/z$ ) calcd. for  $\text{C}_{32}\text{H}_{47}\text{N}_4\text{O}_6\text{S}$   $[\text{M} + \text{H}]^+$ : 615.3211, found: 615.3234. HPLC purity (TFA): >99.0%. Mp 100–104 °C. IR (neat) 3420, 3349, 3010, 2974, 1708, 1618, 1546 (strong), 1452, 1366, 1242, 1163, 1104 (strong), 845, 609  $\text{cm}^{-1}$ . Chiral HPLC: 18.11 minutes (minor) and 23.78 minutes (major) gave an enantiomeric excess of 99%.

### General synthesis of protected dipeptide benzyl ketones (A)

The arginyl benzyl ketone building block **3.15** (3.86 mmol, 1.00 equiv) was dissolved in 20% TFA in DCM (30 mL). The reaction mixture was stirred at room temperature for 30 minutes upon which it became slightly colored. LC-MS confirmed full conversion. The mixture was diluted with DCM (10 mL), evaporated to dryness *in vacuo* and the residue was coevaporated with DCM ( $3 \times 10$  mL) affording the free amine (TFA-salt) as a viscous colored oil.

Meanwhile, a *N*-Boc protected amino acid (4.24 mmol, 1.10 equiv) was dissolved in DCM (30 mL) and HOAt (577 mg, 4.24 mmol, 1.10 equiv) and EDC·HCl (813 mg, 4.24 mmol, 1.10 equiv) were added. The reaction mixture was stirred at room temperature for 30 minutes which afforded the activated amino acid.

The free amine (TFA-salt) was redissolved in DCM (30 mL) and the mixture was added to the stirred solution of the activated amino acid. pH was adjusted to 5–6 by slowly addition of DIPEA (approx. 1–2 mL). The reaction mixture became instantly yellow. The mixture was stirred at room temperature for 1 hour at which time LC-MS showed full conversion (>95%). The reaction mixture was diluted with DCM (300 mL) and washed with 0.5M HCl ( $2 \times 200$  mL), sat. aqueous  $\text{NaHCO}_3$  ( $2 \times 200$  mL) and finally with brine ( $2 \times 200$  mL). The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated to dryness *in vacuo*. Purification by flash chromatography afforded the protected dipeptide benzyl ketone.

### Boc-Thr(Bn)-Arg(Pmc)-bk (3.16)

Following the general synthesis of protected dipeptide benzyl ketones (A) with Boc-Thr(Bn)-OH and Boc-Arg(Pmc)-bk (**3.15**) as starting materials and purification by flash chromatography (60→100% EtOAc in heptane) afforded the product as a white foam (2.09 g, 67%).  $R_f = 0.24$  (80% EtOAc in heptane). HRMS ( $m/z$ ) calcd. for  $\text{C}_{43}\text{H}_{60}\text{N}_5\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$ : 806.4157, found: 806.4192. HPLC purity (TFA): >96.4%.

### Boc-Leu-Arg(Pmc)-bk (3.17)

Following the general synthesis of protected dipeptide benzyl ketones (**A**) with Boc-Leu-OH and Boc-Arg(Pmc)-bk (**3.15**) as starting materials and purification by flash chromatography (60→100% EtOAc in heptane) afforded the product as a pale, white foam (1.29 g, 62%). HRMS ( $m/z$ ) calcd. for  $C_{38}H_{58}N_5O_7S$   $[M + H]^+$ : 728.4052, found: 728.4060. HPLC purity (TFA): >94.8%.

### Boc-Phe-Arg(Pmc)-bk (**3.18**)

Following the general synthesis of protected dipeptide benzyl ketones (**A**) with Boc-Phe-OH and Boc-Arg(Pmc)-bk (**3.15**) as starting materials and purification by flash chromatography (40→80% EtOAc in heptane) afforded the product as a white foam (1.54 g, 60%).  $R_f = 0.51$  (80% EtOAc in heptane).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  7.30–7.11 (m, 10H), 6.26 (s, 2H), 6.16 (broad s, 1H), 5.34 (d,  $J = 5.6$  Hz, 1H), 4.56 (m, 1H), 4.45 (m, 1H), 3.72–3.63 (m, 2H), 3.13–3.06 (m, 3H), 2.93–2.88 (m, 1H), 2.63–2.61 (m, 2H), 2.58 (d,  $J = 8.8$  Hz, 6H), 2.11 (s, 3H), 1.94 (s, 1H), 1.80–1.77 (m, 2H), 1.65–1.51 (m, 1H), 1.51–1.39 (m, 2H), 1.32 (s, 9H), 1.30 (s, 6H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  206.1, 172.3, 156.1, 153.6, 136.4, 135.5, 134.8, 133.3, 133.2, 129.6, 129.2, 128.6, 128.5, 127.1, 126.9, 124.1, 117.9, 80.2, 73.6, 60.4, 57.3, 55.9, 46.3, 40.6, 38.2, 32.8, 28.2, 26.7, 25.0, 21.4, 18.6, 17.5, 12.1. HRMS ( $m/z$ ) calcd. for  $C_{41}H_{56}N_5O_7S$   $[M + H]^+$ : 762.3895, found: 762.3922. HPLC purity (TFA): >91.7%.

### General synthesis of protected tripeptide benzyl ketones (**B**)

The protected dipeptide benzyl ketone (1.79 mmol, 1.00 equiv) was dissolved in 20% TFA in DCM (30 mL) at 0 °C and stirred for up to 4 hours upon which the mixture became slightly colored. LC-MS confirmed full conversion. The reaction mixture was diluted with DCM (10 mL), evaporated to dryness *in vacuo* and the residue was coevaporated with DCM (3 × 10 mL) affording the free amine (TFA-salt) as a viscous colored oil.

Meanwhile, a *N*-Boc protected amino acid (1.97 mmol, 1.10 equiv) was dissolved in DCM (30 mL) and HOAt (268 mg, 1.97 mmol, 1.10 equiv) and EDC·HCl (378 mg, 1.97 mmol, 1.10 equiv) were added. The reaction mixture was stirred at room temperature for 30 minutes and then cooled to 0 °C.

The free amine (TFA-salt) was redissolved in DCM (30 mL) and the mixture was added to the stirred solution of the activated amino acid at 0 °C. pH was adjusted to 5–6 by slowly addition of DIPEA (1–2 mL). The reaction mixture became instantly yellow. The mixture was stirred at 0 °C for 1 hour at which time LC-MS showed full conversion (>95%). The reaction mixture was diluted with DCM (300 mL) and washed with 0.5M HCl (2 × 200 mL), sat. aqueous  $NaHCO_3$  (2 × 200 mL) and finally with brine (2 × 200 mL). The organic layer was dried over anhydrous  $Na_2SO_4$  and evaporated to dryness *in vacuo*. Purification by flash chromatography afforded the protected tripeptide benzyl ketone.

### Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**)

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Boc-Tyr(Bn)-OH and Boc-Thr(Bn)-Arg(Pmc)-bk (**3.16**) as starting materials and purification by flash chromatography (25 → 90% EtOAc in heptane), afforded the product as pale, yellow foam (1.56 g, 62%).  $R_f = 0.43$  (95% EtOAc in heptane). HRMS ( $m/z$ ) calcd. for  $C_{59}H_{75}N_6O_{10}S$   $[M + H]^+$ : 1059.5260, found: 1059.5304. HPLC purity (TFA): >69.3%.

#### **Boc-Tyr(Bn)-Leu-Arg(Pmc)-bk (3.20)**

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Boc-Tyr(Bn)-OH and Boc-Leu-Arg(Pmc)-bk (**3.17**) as starting materials and purification by flash chromatography (25→90% EtOAc in heptane), afforded the product as pale, yellow solid (339 mg, 39%).  $R_f = 0.65$  (EtOAc). HRMS ( $m/z$ ) calcd. for  $C_{54}H_{73}N_6O_9S$   $[M + H]^+$ : 981.5154, found: 981.5202. HPLC purity (TFA): >79.2%.

#### **Boc-Phe-Leu-Arg(Pmc)-bk (3.21)**

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Boc-Phe-OH and Boc-Leu-Arg(Pmc)-bk (**3.17**) as starting materials and purification by flash chromatography (75→100% EtOAc in heptane), afforded the product as white solid (224 mg, 29%).  $R_f = 0.42$  (90% EtOAc in heptane). HRMS ( $m/z$ ) calcd. for  $C_{47}H_{67}N_6O_8S$   $[M + H]^+$ : 875.4736, found: 875.4762. HPLC purity (TFA): >85.2%.

#### **Boc-Phe-Phe-Arg(Pmc)-bk (3.22)**

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Boc-Phe-OH and Boc-Phe-Arg(Pmc)-bk (**3.18**) as starting materials and purification by flash chromatography (75→100% EtOAc in heptane) afforded the product as a white foam (882 mg, 54%).  $R_f = 0.51$  (80% EtOAc in heptane). MS ( $m/z$ ) calcd. for  $C_{50}H_{65}N_6O_8S$   $[M + H]^+$ : 909.5, found: 909.9. HPLC purity (TFA): >88.5%.

#### **Boc-D-Phe-Phe-Arg(Pmc)-bk (3.23)**

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Boc-D-Phe-OH and Boc-Phe-Arg(Pmc)-bk (**3.18**) as starting materials and purification by flash chromatography (75→100% EtOAc in heptane) afforded the product as a white foam (237 mg, 27%). MS ( $m/z$ ) calcd. for  $C_{50}H_{65}N_6O_8S$   $[M + H]^+$ : 909.5, found: 909.8. HPLC purity (TFA): >79.6%.

**Cbz-Phe-Phe-Arg(Pmc)-bk (3.24)**

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Cbz-Phe-OH and Boc-Phe-Arg(Pmc)-bk (**3.18**) as starting materials and purification by flash chromatography (25→90% EtOAc in heptane), afforded the product as a pale, yellow glass (157 mg, 32%).  $R_f = 0.73$  (EtOAc). HRMS ( $m/z$ ) calcd. for  $C_{53}H_{63}N_6O_8S$   $[M + H]^+$  943.4423, found 943.4465. HPLC purity (TFA): >69.3%.

**Cbz-D-Phe-Phe-Arg(Pmc)-bk (3.25)**

Following the general synthesis of protected tripeptide benzyl ketones (**B**) with Cbz-D-Phe-OH and Boc-Phe-Arg(Pmc)-bk (**3.18**) as starting materials and purification by flash chromatography (25→90% EtOAc in heptane), afforded the product as a pale, yellow glass (133 mg, 27%). HRMS ( $m/z$ ) calcd. for  $C_{53}H_{63}N_6O_8S$   $[M + H]^+$  943.4423, found 943.4458. HPLC purity (TFA): >80.6%.

**Ac-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (3.26)**

Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**) (86 mg, 0.08 mmol, 1.00 equiv) was dissolved in 20% TFA in DCM (5 mL) at room temperature and stirred for 30 minutes. Then diluted with DCM (10 mL) and evaporated *in vacuo* and coevaporated to dryness *in vacuo* with DCM (3 × 10 mL). The unprotected tripeptide (TFA-salt) was dissolved in DCM (7 mL) and DIPEA (44  $\mu$ L, 31 mg, 0.24 mmol, 3.00 equiv) and  $Ac_2O$  (22  $\mu$ L, 21 mg, 0.20 mmol, 2.50 equiv) were slowly added while stirring. pH adjusted to 7–8 by addition of DIPEA. Stirring was continued at room temperature for 30 minutes, then diluted with DCM (25 mL) and washed with 1.0M HCl (2 × 25 mL), sat. aqueous  $NaHCO_3$  (2 × 25 mL) and brine (2 × 25 mL). The organic phase was dried over anhydrous  $Na_2SO_4$  and evaporated to dryness *in vacuo*. Purification by flash chromatography (0→15% MeOH in DCM) afforded the *N*-acylated tripeptide **3.26** as a pale yellow glass (54 mg, 67% (2 steps)).  $R_f = 0.37$  (5% MeOH in DCM). MS ( $m/z$ ) calcd. for  $C_{56}H_{69}N_6O_9S$   $[M + H]^+$ : 1001.5, found: 1001.8. HPLC purity (TFA): >92.1%.

**Ac-Phe-Phe-Arg(Pmc)-bk (3.27)**

Boc-Phe-Phe-Arg(Pmc)-bk (**3.22**) (250 mg, 0.28 mmol, 1.00 equiv) was dissolved in 20% TFA in DCM (10 mL) at room temperature and stirred for 30 minutes. Then diluted with DCM (10 mL) and evaporated to dryness *in vacuo* and the residue was coevaporated with DCM (3 × 10 mL). The unprotected tripeptide (TFA-salt) was dissolved in DCM (25 mL) and  $Ac_2O$  (75  $\mu$ L, 70 mg, 0.69 mmol, 2.50 equiv) was added slowly while stirring. pH was adjusted to 7–8 by addition of DIPEA. Stirring was continued at room temperature for 30 minutes, then diluted with DCM (50 mL) and washed with 1.0M HCl (2 × 50 mL), sat. aqueous  $NaHCO_3$  (2 × 50 mL) and brine (2 × 50 mL). The organic phase was dried over anhydrous  $Na_2SO_4$  and evaporated to dryness *in vacuo*. Purification by flash chromatography (5→20% MeOH in DCM) afforded the *N*-acylated tripeptide **3.27** as a pale, yellow solid (165 mg, 70% (2 steps)).  $R_f = 0.61$



(10% MeOH in DCM). MS ( $m/z$ ) calcd. for  $C_{47}H_{59}N_6O_7S$   $[M + H]^+$ : 851.4, found: 851.7. HPLC purity (TFA): >89.6%.

### General *N*-sulfonylation of tripeptides (C)

The protected tripeptide (0.28 mmol, 1.00 equiv) was dissolved in 20% TFA in DCM (10 mL) and stirred for 30 minutes at room temperature. The reaction mixture was diluted with DCM (10 mL) and evaporated to dryness *in vacuo*. The residue was then coevaporated with DCM ( $3 \times 15$  mL). The free amine (TFA-salt) was dissolved in DCM (25 mL) and  $BnSO_2Cl$  (58 mg, 0.30 mmol, 1.10 equiv) was added at once and pH was adjusted to 7–8 by addition of DIPEA. The reaction mixture was stirred at room temperature until full conversion was observed on LC-MS (if ever observed). The reaction mixture was diluted with DCM (50 mL) and washed with 1M HCl ( $2 \times 50$  mL), sat. aqueous  $NaHCO_3$  ( $2 \times 50$  mL) and brine ( $2 \times 50$  mL). The organic layer was dried over anhydrous  $Na_2SO_3$  and evaporated to dryness *in vacuo*. The crude product was purified by flash chromatography, either by 0→20% MeOH in DCM or by heptane→EtOAc, but none of the systems were able to remove all impurities. Therefore some of the products were used without further purification.

### $BnSO_2$ -Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (3.28)

Following the general *N*-sulfonylation of tripeptides (C) with Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (3.19) as starting material afforded the product as a pale, yellow solid (223 mg, 23%).  $R_f = 0.73$  (EtOAc). HRMS ( $m/z$ ) calcd. for  $C_{61}H_{73}N_6O_{10}S_2$   $[M + H]^+$ : 1113.4824, found: 1113.4873. HPLC purity (TFA): >77.6%.

### $BnSO_2$ -Tyr(Bn)-Leu-Arg(Pmc)-bk (3.29)

Following the general *N*-sulfonylation of tripeptides (C) with Boc-Tyr(Bn)-Leu-Arg(Pmc)-bk (3.20) as starting material afforded the product which was used without further purification. MS ( $m/z$ ) calcd. for  $C_{56}H_{71}N_6O_9S_2$   $[M + H]^+$ : 1035.5, found: 1035.6. HPLC purity (TFA): >59.9%.

### $BnSO_2$ -Phe-Leu-Arg(Pmc)-bk (3.30)

Following the general *N*-sulfonylation of tripeptides (C) with Boc-Phe-Leu-Arg(Pmc)-bk (3.21) as starting material and purification by flash chromatography (0→20% MeOH in DCM) affording the desired product as a pale, yellow solid (164 mg, 74%).  $R_f = 0.46$  (5% MeOH in DCM). MS ( $m/z$ ) calcd. for  $C_{49}H_{65}N_6O_8S_2$   $[M + H]^+$ : 929.4, found: 929.4. HPLC purity (TFA): >50.8%.

**BnSO<sub>2</sub>-Phe-Phe-Arg(Pmc)-bk (3.31)**

Following the general *N*-sulfonylation of tripeptides (**C**) with Boc-Phe-Phe-Arg(Pmc)-bk (**3.22**) as starting material (reaction time: 16 hours and additional 0.5 equiv BnSO<sub>2</sub>Cl was added) and purification by flash chromatography (25→100% EtOAc in heptane) afforded the product as a white solid (84 mg, 32%). *R<sub>f</sub>* = 0.43 (5% MeOH in DCM). MS (*m/z*) calcd. for C<sub>52</sub>H<sub>63</sub>N<sub>6</sub>O<sub>8</sub>S<sub>2</sub> [M + H]<sup>+</sup>: 963.4, found: 963.9. HPLC purity (TFA): >63.5%.

**BnSO<sub>2</sub>-D-Phe-Phe-Arg(Pmc)-bk (3.32)**

Following the general *N*-sulfonylation of tripeptides (**C**) with Boc-D-Phe-Phe-Arg(Pmc)-bk (**3.23**) as starting material afforded the desired product, which was used without further purification. MS (*m/z*) calcd. for C<sub>52</sub>H<sub>63</sub>N<sub>6</sub>O<sub>8</sub>S<sub>2</sub> [M + H]<sup>+</sup>: 963.4, found: 963.7. HPLC purity (TFA): >39.1%.

**(2S)-3-Phenyl-2-(phenylmethanesulfonylamido)propanoic acid (3.34)<sup>432</sup>**

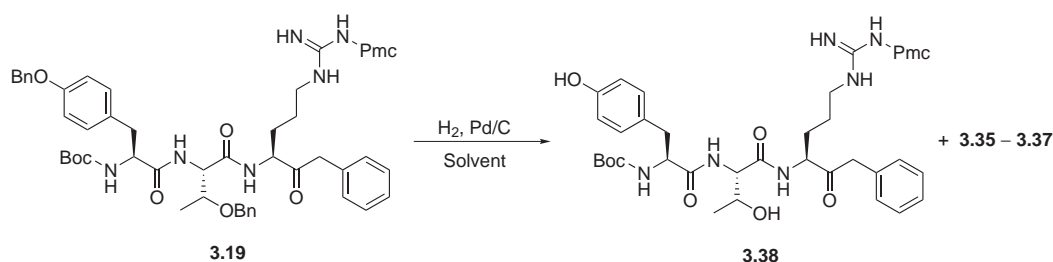
A solution of H-Phe-OH (**3.33**) (3.77 g, 22.81 mmol, 1.00 equiv) was dissolved in 1M NaOH (30 mL) and dioxane (30 mL). Then 1M NaOH (50 mL) and BnSO<sub>2</sub>Cl (5.00 g, 26.23 mmol, 1.50 equiv) in dioxane (50 mL) was added simultaneously in approx. 10 equal portions. Between each addition the mixture was stirred vigorously for 15 minutes at 0 °C. After the final addition, the reaction mixture was stirred for 2 hours. The crude product was washed with Et<sub>2</sub>O (3 × 50 mL) and the aqueous layer was acidified with conc. HCl to pH 1 and sat. with NaCl. Extraction with EtOAc (3 × 50 mL) gave an organic phase which was added heptane (50 mL) and extracted with sat. aqueous NaHCO<sub>3</sub> (3 × 50 mL). The combined aqueous layers were evaporated *in vacuo* to remove the remaining EtOAc, and finally it was acidified by slowly addition of conc. HCl. The product precipitated as an oil, which was extracted with Et<sub>2</sub>O (3 × 50 mL) and the product was obtained by evaporation to dryness *in vacuo* giving a white solid (2.48 g, 35%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 12.85 (broad s, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.34–7.22 (m, 8H), 7.15 (d, *J* = 5.3 Hz, 2H), 4.07–4.04 (m, 1H), 4.02–3.99 (m, 1H), 3.97–3.94 (m, 1H), 3.00 (dd, *J* = 13.5, 5.7 Hz, 1H), 2.81 (dd, *J* = 13.6, 9.1 Hz, 1H). MS (*m/z*) calcd. for C<sub>16</sub>H<sub>18</sub>NO<sub>4</sub>S [M + H]<sup>+</sup>: 320.1, found: 320.1. Mp 152–158 °C (lit.:<sup>432</sup> 156–157 °C).

**General catalytic hydrogenolysis for O-debenzylation (D)**

O-Benzylated tripeptide (0.38 mmol, 1.00 equiv) was dissolved in MeOH (36 mL) in a pressure-resistant chamber and AcOH (4 mL) was added together with Pd/C (150 mg). The chamber was sealed with a lid connected to a manometer and thoroughly purged with N<sub>2</sub> while stirring. Then, H<sub>2</sub> gas (80 bar) was applied a couple of times for complete replacement of the internal atmosphere. Finally, the chamber was filled with H<sub>2</sub> gas (80 bar) and the mixture was stirred vigorously by placing the reaction chamber on a magnetic stirrer. Stirring was continued for 24 hours at room temperature. The



chamber was then ventilated several times with N<sub>2</sub> before the lid was removed. The black mixture was filtered through a plug of Celite<sup>®</sup> and washed with MeOH (3 × 25 mL). The combined filtrates were evaporated to dryness *in vacuo* and the residue was coevaporated with DCM (3 × 25 mL). The product was used without further purification.



| entry | solvent <sup>a</sup> | P (bar) | temp. (°C) | time (h) | 3.19 <sup>b</sup> | 3.35 <sup>b</sup> | 3.37 <sup>b</sup> | 3.38 <sup>b</sup> |
|-------|----------------------|---------|------------|----------|-------------------|-------------------|-------------------|-------------------|
| 1     | A                    | 3       | rt         | 16       | 43                | 43                | 0                 | 14                |
| 2     | A                    | 80      | rt         | 16       | 0                 | 84                | 0                 | 16                |
| 3     | B                    | 3       | rt         | 16       | 3                 | 97                | 0                 | 0                 |
| 4     | B                    | 40      | 40         | 16       | 0                 | 39                | 22                | 39                |
| 5     | B                    | 80      | 50         | 3        | 0                 | 73                | 0 <sup>c</sup>    | 18                |
| 6     | B                    | 80      | 50         | 16       | 0                 | 0                 | 45 <sup>c</sup>   | 10                |
| 7     | C                    | 40      | rt         | 3        | 52                | 48                | 0                 | 0                 |
| 8     | C                    | 40      | rt         | 5        | 43                | 57                | 0                 | 0                 |
| 9     | C                    | 40      | rt         | 16       | 0                 | 40                | 0                 | 60                |
| 10    | C                    | 80      | rt         | 16       | 0                 | 21                | 5                 | 74                |
| 11    | C                    | 80      | rt         | 24       | 0                 | 6                 | 7                 | 87                |
| 12    | C                    | 80      | rt         | 72       | 0                 | 0                 | 22                | 78                |
| 13    | C                    | 80      | 30         | 16       | 0                 | 13                | 7                 | 80                |
| 14    | C                    | 80      | 35         | 16       | 0                 | 0                 | 26                | 74                |

<sup>a</sup>: A: EtOH, B: MeOH, C: MeOH and 10% AcOH. <sup>b</sup>: Distribution of compounds in the crude product (%), determined by LC-MS, UV 214 nm. <sup>c</sup>: The di O-debenzylated and reduced by-product 3.36 was also formed.

### Boc-Tyr-Thr-Arg(Pmc)-bk (3.38)

Following the general hydrogenolysis for *O*-debenzylation (**D**) with Boc-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**) (400 mg, 0.38 mmol, 1.00 equiv) as starting material gave full conversion (>95%) after 24 hours at room temperature. Only minor amounts of the reduced tyrosine (4%) was present based on LC-MS analysis. MS (*m/z*) calcd. for C<sub>45</sub>H<sub>63</sub>N<sub>6</sub>O<sub>10</sub>S [M + H]<sup>+</sup>: 879.4, found: 879.7. HPLC purity (TFA): >80.1%.

### Ac-Tyr-Thr-Arg(Pmc)-bk (3.39)

Following the general hydrogenolysis for *O*-debenzylation (**D**) with Ac-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.19**) (271 mg, 0.27 mmol, 1.00 equiv) as starting material gave only

50% conversion after 24 hours at room temperature. Therefore, the reaction mixture was once again subjected to H<sub>2</sub> (80 bar) for totally 2.5 days, which then gave full conversion (>95%). Approx. 10% of the reduced tyrosine was also formed. MS (*m/z*) calcd. for C<sub>42</sub>H<sub>57</sub>N<sub>6</sub>O<sub>9</sub>S [M + H]<sup>+</sup>: 821.4, found: 821.7.

#### **BnSO<sub>2</sub>-Tyr-Thr-Arg(Pmc)-bk (3.40)**

Following the general hydrogenolysis for *O*-debenzylation (**D**) with BnSO<sub>2</sub>-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.28**) (224 mg, 0.20 mmol, 1.00 equiv) as starting material did not give any traces of the desired product. After several days only the starting material and the corresponding mono *O*-debenzylated compound was observed by LC-MS.

#### **BnSO<sub>2</sub>-Tyr-Leu-Arg(Pmc)-bk (3.41)**

Following the general hydrogenolysis procedure for *O*-debenzylation (**D**) with BnSO<sub>2</sub>-Tyr(Bn)-Thr(Bn)-Arg(Pmc)-bk (**3.29**) (529 mg, 0.51 mmol, 1.00 equiv) as starting material did not give any traces of the desired product. After several days only the starting material and the corresponding mono *O*-debenzylated compound was observed by LC-MS.

#### **General procedure for deprotection, purification and lyophilization (E)**

The protected tripeptide was dissolved in 95% TFA in DCM (10 mL) at room temperature and stirred for 30 minutes, at which time LC-MS showed full conversion (>95%). The crude product was evaporated to dryness *in vacuo* the residue was coevaporated with DCM (3 × 15 mL). Then dissolved in 10% MeCN in H<sub>2</sub>O (≈ 50 mL) and the insoluble compounds (primarily the cleaved Pmc-group) were removed by filtration through a Corning<sup>®</sup> vacuum filter (0.22 μm polyethersulfone membrane, sterile). The filtrate was purified by reverse-phase preparative HPLC.

#### **H-Tyr-Thr-Arg-bk (3.42)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with Boc-Tyr-Thr-Arg(Pmc)-bk (**3.33**) (332 mg, 0.38 mmol, 1.00 equiv) as starting material and a preparative HPLC purification using a gradient of 13→33% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (67 mg peptide determined by HPLC-CLND, 35% (3 steps). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 9.39 (broad s, 1H), 8.69 (d, *J* = 8.1 Hz, 1H), 8.41 (d, *J* = 7.1 Hz, 1H), 8.02 (broad s, 3H), 7.78–7.72 (m, 1H), 7.31–7.21 (m, 4H), 7.15–7.13 (m, 2H), 7.10–7.08 (m, 2H), 6.70 (d, *J* = 8.3 Hz, 2H), 5.10 (broad s, 1H), 4.36–4.29 (m, 2H), 4.17–4.06 (m, 2H), 3.85 (s, 2H), 3.15–3.05 (m, 3H), 2.79–2.73 (m, 1H), 1.87–1.77 (m, 1H), 1.64–1.54 (m, 1H), 1.54–1.43 (m, 2H), 1.13 (d, *J* = 6.1 Hz, 3H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 206.5, 170.0, 168.6, 158.5, 158.1, 157.8, 156.8, 156.5, 134.6, 130.5, 129.8, 128.1, 126.4, 124.8, 118.6, 115.7, 115.3, 66.8, 57.8, 53.5, 44.6, 36.3, 26.5, 24.9, 19.9. HRMS (*m/z*) calcd. for C<sub>26</sub>H<sub>37</sub>N<sub>6</sub>O<sub>5</sub> [M + H]<sup>+</sup>: 513.2820, found: 513.2840. HPLC purity (TFA): 93.0%, (NH<sub>4</sub>HCO<sub>3</sub>): 98.7%.

**Ac-Tyr-Thr-Arg-bk (3.43)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with Ac-Tyr-Thr-Arg(Pmc)-bk (**3.34**) (222 mg, 0.27 mmol, 1.00 equiv) as starting material and a preparative HPLC purification using a gradient of 15→35% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (22 mg peptide determined by HPLC-CLND, 14% (3 steps). An equivalent portion was also collected but that contained 22% of the reduced tyrosine (determined by HPLC). HRMS (*m/z*) calcd. for C<sub>28</sub>H<sub>39</sub>N<sub>6</sub>O<sub>6</sub> [M + H]<sup>+</sup>: 555.2926, found: 555.2951. HPLC purity (TFA): 89.3%, (NH<sub>4</sub>HCO<sub>3</sub>): 90.9%, (Na<sub>2</sub>SO<sub>3</sub>): 87.9%.

**BnSO<sub>2</sub>-Phe-Leu-Arg-bk (3.44)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with BnSO<sub>2</sub>-Tyr-Thr-Arg(Pmc)-bk (**3.35**) (350 mg, 0.38 mmol, 1.00 equiv) as starting material and a preparative HPLC purification using a gradient of 28→48% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (75 mg peptide determined by HPLC-CLND, 30% (2 steps)). HRMS (*m/z*) calcd. for C<sub>35</sub>H<sub>47</sub>N<sub>6</sub>O<sub>5</sub>S [M + H]<sup>+</sup>: 663.3323, found: 663.3338. HPLC purity (TFA): 97%, (Na<sub>2</sub>SO<sub>3</sub>): 95.3%.

**H-Phe-Phe-Arg-bk (3.45)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with Boc-Phe-Phe-Arg(Pmc)-bk (**3.22**) as starting material and a preparative HPLC purification using a gradient of 20→40% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (58 mg peptide determined by HPLC-CLND, 49% (2 steps)). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.94 (d, *J* = 7.8 Hz, 1H), 8.76 (d, *J* = 7.1 Hz, 1H), 8.10 (broad s, 3H), 7.84–7.80 (m, 1H), 7.29–7.16 (m, 15H), 7.09 (d, *J* = 7.6 Hz, 2H), 4.71–4.66 (m, 1H), 4.29–4.24 (m, 1H), 4.04 (broad s, 1H), 3.70–3.57 (m, 4H), 3.15–3.03 (m, 4H), 2.93–2.86 (m, 2H), 1.83–1.75 (m, 1H), 1.58–1.48 (m, 1H), 1.44–1.38 (m, 2H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 206.3, 170.8, 168.1, 158.9, 158.6, 158.3, 158.0, 156.9, 137.1, 134.7, 134.5, 129.7, 129.5, 129.2, 128.5, 128.2, 128.1, 127.2, 126.6, 126.5, 118.6, 115.6, 57.9, 54.1, 53.1, 44.6, 37.7, 37.0, 26.4, 25.0. HRMS (*m/z*) calcd. for C<sub>31</sub>H<sub>39</sub>N<sub>6</sub>O<sub>3</sub> [M + H]<sup>+</sup>: 543.3078, found 543.3099. HPLC purity (TFA): 95.4%, (NH<sub>4</sub>HCO<sub>3</sub>): 100%, (Na<sub>2</sub>SO<sub>3</sub>): 97.8%.

**Ac-Phe-Phe-Arg-bk (3.46)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with Ac-Phe-Phe-Arg(Pmc)-bk (**3.27**) (133 mg) as starting material and a preparative HPLC purification using a gradient of 20→40% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (52 mg peptide determined by HPLC-CLND, 57% (2 steps)). HRMS (*m/z*) calcd. for C<sub>33</sub>H<sub>41</sub>N<sub>6</sub>O<sub>4</sub> [M + H]<sup>+</sup> 585.3184, found 585.3205. HPLC purity (TFA): 95.9%, (NH<sub>4</sub>HCO<sub>3</sub>): 98.8%, (Na<sub>2</sub>SO<sub>3</sub>): 95.9%.

**BnSO<sub>2</sub>-Phe-Phe-Arg-bk (3.47)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with BnSO<sub>2</sub>-Phe-Phe-Arg(Pmc)-bk (**3.31**) (84 mg) as starting material and a preparative HPLC purification using a gradient of 25→45% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (15 mg peptide determined by HPLC-CLND, 24% (3 steps)). HRMS (*m/z*) calcd. for C<sub>38</sub>H<sub>45</sub>N<sub>6</sub>O<sub>5</sub>S [M + H]<sup>+</sup> 697.3167, found 697.3183. HPLC purity (TFA): 94.5%, (NH<sub>4</sub>HCO<sub>3</sub>): 96.9%, (Na<sub>2</sub>SO<sub>3</sub>): 97.2%.

**BnSO<sub>2</sub>-D-Phe-Phe-Arg-bk (3.48)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with BnSO<sub>2</sub>-D-Phe-Phe-Arg(Pmc)-bk (**3.32**) (240 mg) as starting material and a preparative HPLC purification using a gradient of 25→45% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (51 mg determined by HPLC-CLND, 29%). HRMS (*m/z*) calcd. for C<sub>38</sub>H<sub>45</sub>N<sub>6</sub>O<sub>5</sub>S [M + H]<sup>+</sup> 697.3167, found 697.3175. HPLC purity (TFA): 95.4%, (Na<sub>2</sub>SO<sub>3</sub>): 85.0%.

**Cbz-Phe-Phe-Arg-bk (3.49)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with Cbz-Phe-Phe-Arg(Pmc)-bk (**3.24**) (150 mg) as starting material and a preparative HPLC purification using a gradient of 28→48% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (23 mg determined by HPLC-CLND, 21% (2 steps)). HRMS (*m/z*) calcd. for C<sub>39</sub>H<sub>45</sub>N<sub>6</sub>O<sub>5</sub> [M + H]<sup>+</sup> 677.3446, found 677.3435. HPLC purity (TFA): 97.4%, (Na<sub>2</sub>SO<sub>3</sub>): 74.3%.

**Cbz-D-Phe-Phe-Arg-bk (3.50)**

Following the general procedure for deprotection, purification and lyophilization (**E**) with Cbz-D-Phe-Phe-Arg(Pmc)-bk (**3.25**) (91 mg) as starting material and a preparative HPLC purification using a gradient of 28→48% MeCN in H<sub>2</sub>O. Upon lyophilization the product was afforded as a white TFA-salt (20 mg determined by HPLC-CLND, 22% (2 steps)). HRMS (*m/z*) calcd. for C<sub>39</sub>H<sub>45</sub>N<sub>6</sub>O<sub>5</sub> [M + H]<sup>+</sup> 677.3446, found 677.3443. HPLC purity (TFA): 99.4%, (Na<sub>2</sub>SO<sub>3</sub>): 83.4%.

**General procedures - Biology**

Chemicals and reagents were all commercially available and used without further purifi-

cation. FVIIa (1.39 mg/mL solution in GlyGly buffer at pH 6.0 stored at  $-80^{\circ}\text{C}$ ), TF (sTF<sub>1-219</sub>) (0.91 mg/mL solution, approx.  $36.7\ \mu\text{M}$ , stored at  $-20^{\circ}\text{C}$ ) and thrombin (20 U/mL,  $20\ \mu\text{L}$  stored at  $-80^{\circ}\text{C}$ ) were obtained in-house at Novo Nordisk A/S. hFXa (100 U) was purchased from Enzyme Research, USA. A HEPES stock buffer was prepared by dissolving HEPES (29.79 g, 125 mmol), NaCl (14.61 g, 250 mmol),  $\text{CaCl}_2$  (1.84 g, 16.6 mmol) and Tween80 (0.25 g) in  $\text{H}_2\text{O}$  to a total volume of 250 mL. pH was adjusted to 7.4 and the buffer was filtered through a  $0.22\ \mu\text{m}$  cellulose acetate sterilizing, low protein binding membrane purchased from Corning Incorporated. The stock buffer was stored at  $5^{\circ}\text{C}$  (up to 3 months). The actual assay buffer (50 mM HEPES, 100 mM NaCl, 6.6 mM  $\text{CaCl}_2$ , 0.01% Tween80) was prepared immediately prior to use by diluting the stock buffer 10 times with  $\text{H}_2\text{O}$ . Inhibitor solutions were made by dissolving approx. 8 mg lyophilized inhibitor in a few drops of DMSO and then diluting with assay buffer (5.0 mL). For those compounds showing poor solubility in the buffer, further dissolution was made to get appropriate solubilities. Inhibitor solutions of no more than approx.  $1500\ \mu\text{M}$  were prepared. The concentration of each inhibitor solution was determined by HPLC-CLND. Chromogenic substrates (S-2288, S-2238 and S-2765) were purchased from Chromogenix, Italy, and dissolved in  $\text{H}_2\text{O}$  prior to use. The assays were run in a 96-well assay plate. The chromogenic substrate was added at last just immediately before absorbance reading at a SpectraMax M2<sup>e</sup> Molecular Devices plate reader. The OD<sub>405</sub> (absorbance at 405 nm) was monitored over the course of 15 minutes with 40 second interval readings (assay plate was automatically shaken). The OD<sub>405</sub> was plotted against time and the enzyme velocity ( $v_n$ ) was calculated as the slope of the curve, which was linear with the tested enzyme concentration and within the time frame of 15 minutes. Initial screenings were performed using the highest available inhibitor concentration for estimation of IC<sub>50</sub> values. Only those compounds showing more than 80% inhibition of the enzyme at the highest tested inhibitor concentration, was taken to a  $K_i$  determination.  $K_i$  values were determined by varying the inhibitor concentration, ideally at least 10 times higher and 10 times lower than  $K_i$  with a constant concentration of enzyme and chromogenic substrate. Linear fitting of  $(v_0/v_n)-1$  versus  $[I]$  gave  $K_i = 1/\text{slope}$ , where  $v_0$  is the enzyme velocity in the absence of inhibitor and  $v_n$  is enzyme velocity (OD<sub>405</sub> per time unit) at a given inhibitor concentration  $[I]$ . All results were measured as triplicates and the final  $K_i$  value was calculated as an average.

### **FVIIa competitive binding assay**

FVIIa and TF were thawed to  $0^{\circ}\text{C}$  on ice prior to use. FVIIa was diluted to a 200 nM concentration ( $10\ \mu\text{L}$  FVIIa stock solution was diluted with  $490\ \mu\text{L} + 900\ \mu\text{L}$  assay buffer affording a  $9.93\ \mu\text{g/mL}$  concentration,  $M_w \approx 50,000\ \text{g/mol}$ ). TF was also diluted to a 200 nM concentration ( $20\ \mu\text{L}$  TF stock solution was diluted with  $980\ \mu\text{L} + 2662\ \mu\text{L}$  assay buffer affording a  $4.97\ \mu\text{g/mL}$  concentration). S-2288 (H-D-Ile-Pro-Arg-pNA, 25 mg) was dissolved in  $\text{H}_2\text{O}$  (1.0 mL) giving a stock concentration of 43.3 mM. In a 96-well assay plate was mixed FVIIa ( $10\ \mu\text{L}$ ), TF ( $50\ \mu\text{L}$ ), assay buffer and/or inhibitor

solution (135  $\mu$ L) and finally the assay was started by addition of S-2288 (5  $\mu$ L) giving a total volume of 200  $\mu$ L in each well with final concentrations as following: FVIIa: 10 nM, TF: 50 nM and S-2288: 1 mM and varying inhibitor concentrations. The plate was immediately monitored at OD<sub>405</sub> as described above.

### **Thrombin (thr) competitive binding assay**

Thrombin was thawed to 0 °C on ice prior to use, and then diluted to a 0.4 U/mL activity (480  $\mu$ L buffer + 20  $\mu$ L thr). S-2238 (H-D-Phe-Pip-Arg-pNA, 25 mg) was dissolved in H<sub>2</sub>O (2.5 mL) giving a stock concentration of 16 mM. In a 96-well assay plate was mixed thrombin (5  $\mu$ L), assay buffer and/or inhibitor solution (185  $\mu$ L) and finally the assay was started by addition of S-2238 (10  $\mu$ L) giving a total volume of 200  $\mu$ L in each well with final concentrations of following: thr: 20 mU/mL and S-2238: 400  $\mu$ M and varying inhibitor concentrations). The plate was immediately monitored at OD<sub>405</sub> as described above.

### **FXa competitive binding assay**

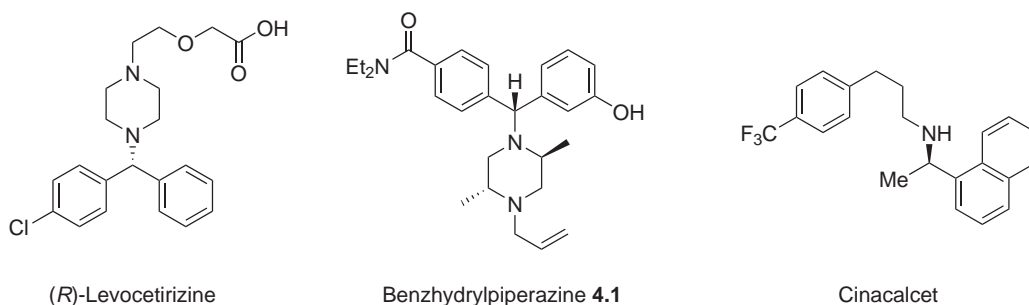
hFXa was dissolved and diluted in the assay buffer to a 0.1 U/mL activity (hFXa was dissolved in 1000  $\mu$ L assay buffer. 5  $\mu$ L of this solution was diluted with 4995  $\mu$ L assay buffer). S-2765 (Cbz-D-Arg-Gly-Arg-pNA, 25 mg) was dissolved in H<sub>2</sub>O (2.0 mL) giving a stock concentration of 17 mM. In a 96-well assay plate was mixed hFXa (15  $\mu$ L), assay buffer and/or inhibitor solution (180  $\mu$ L) and finally the assay was started by addition of S-2765 (5  $\mu$ L) affording a total volume of 200  $\mu$ L in each well with final concentrations as following: hFXa: 5.0 mU/mL and S-2765: 436  $\mu$ M and varying inhibitor concentrations). The plate was immediately monitored at OD<sub>405</sub> as described above.

## Chapter 4

# Rh-Catalyzed Enantioselective Synthesis of Diaryl Amines

### 4.1 Biologically active aryl amines

The majority of small-molecule drugs and drug candidate contains an amine functionality. Therefore asymmetric synthesis of amines is of great importance in medicinal chemistry. With the exception of  $\alpha$ -amino acids, general asymmetric syntheses of  $\alpha$ -branched amines were previously only little explored. Therefore, Professor Jonathan Ellman and his group at the University of California at Berkeley has investigated this area for more than a decade.

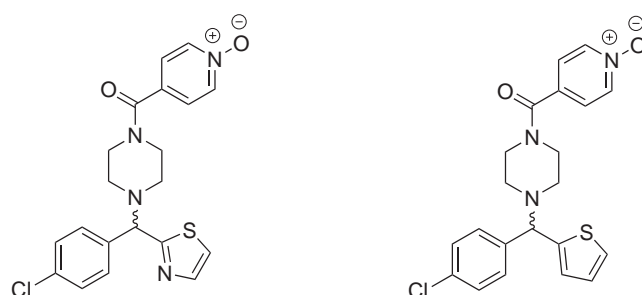


**Figure 4.1.1:** Examples of biologically active aryl amines.<sup>438–442</sup>

Since the late 1940s diarylmethyl piperazines have been of considerable interest due to their antihistaminic properties.<sup>443,444</sup> During the 1980s, racemic cetirizine (formulated as the dihydrochloride salt) was developed by UCB Pharma as a second generation antihistamine.<sup>445</sup> In Europe it was launched in the late 1980s as Zyrtec<sup>®</sup> and became a

blockbuster. The active (*R*)-enantiomer of cetirizine<sup>a</sup>, levocetirizine, was launched in Europe in 2001 and in the US in 2007,<sup>447</sup> as a third generation antihistaminic agent under the trademark of Xyzal<sup>®</sup> (see Figure 4.1.1). Levocetirizine is a non-sedating histamine H<sub>1</sub>-receptor antagonist and is used for the treatment of allergies, such as allergic rhinitis, conjunctivitis, pruritus and urticaria. Levocetirizine is administrated in only half the dose (5 mg) compared to the racemic cetirizine, and it has a twofold higher affinity for the human H<sub>1</sub>-receptor.<sup>439,449</sup> Thus, it is believed that Xyzal<sup>®</sup> has fewer side effects than Zyrtec<sup>®</sup>.

Other aryl amines with interesting pharmacological properties have been reported. Benzhydrylpiperazine **4.1** has been found to act as a potent  $\delta$ -opioid receptor agonist (analgesic)<sup>440,441</sup> and the arylalkyl amine, cinacalcet (see Figure 4.1.1), as an allosteric modulator of the CaR (calcium-sensing receptor). Cinacalcet has recently been approved for the treatment of secondary hyperparathyroidism.<sup>442</sup> The importance of this class of amines is also demonstrated by the many compounds claimed in the patent literature, e.g. patent protection of pyridine *N*-oxide derivatives of diarylmethyl piperazines reported by Schering Corporation as PAF (platelet activating factor) antagonists and antihistamines (see Figure 4.1.2 for examples of two heterocycle derivatives).<sup>450</sup>



**Figure 4.1.2:** Pyridine *N*-oxide derivatives of diarylmethyl piperazines are potential PAF antagonists and antihistamines.<sup>450</sup> Claimed and synthesized in a patent from 1995 by Schering Corporation.

<sup>a</sup>Sepracor<sup>110,446</sup> has incorrectly reported that levocetirizine is the (*S*)-enantiomer. The correct configuration is (*R*)-(-), which is confirmed by several publications, e.g. from UCB Pharma,<sup>439</sup> FDA<sup>447</sup> and in a review on stereoconfiguration of antiallergic drugs from 2008.<sup>448</sup>

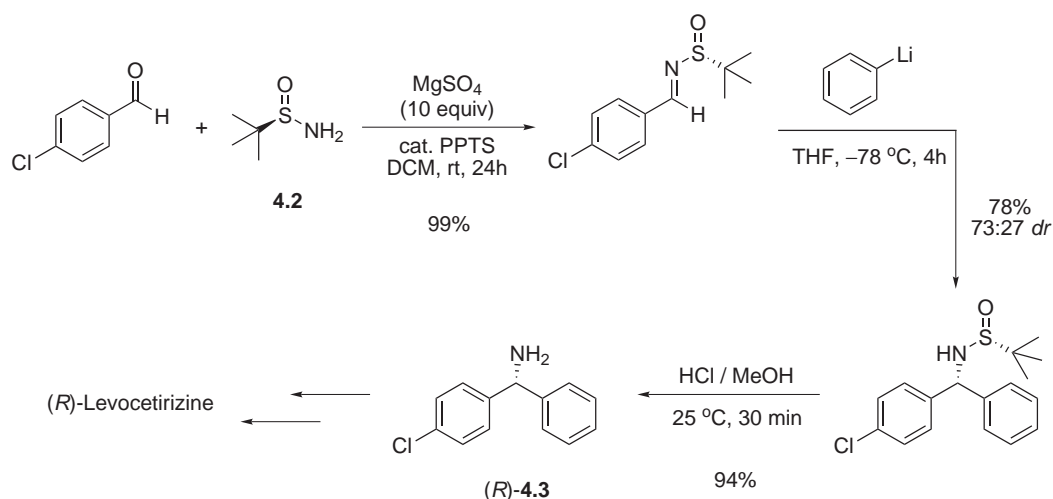


## 4.2 Synthesis of aryl amines

### 4.2.1 Auxiliary-based syntheses

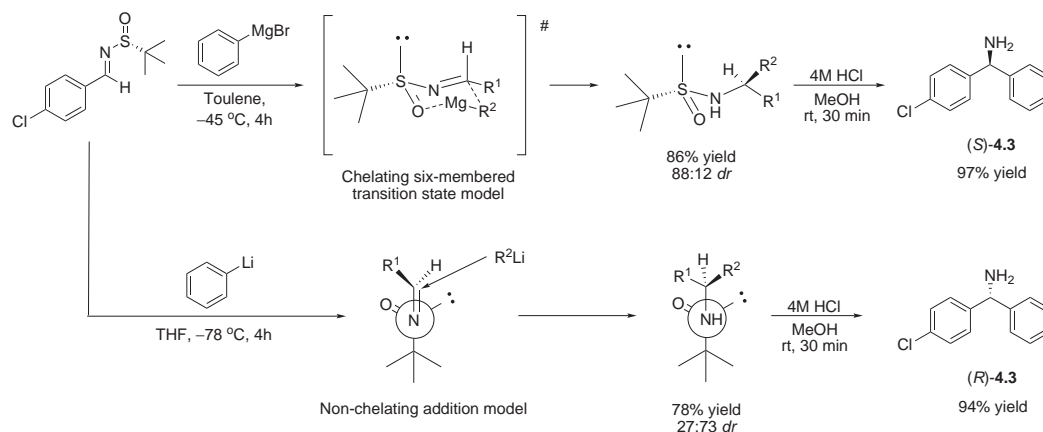
In 1997 the Ellman group reported the first use of (*R*)-*tert*-butanesulfinamide (**4.2**) as a chiral auxiliary for the asymmetric synthesis of  $\alpha$ - and  $\alpha,\alpha$ -dibranched amines.<sup>451,452</sup> This method utilizes a 1,2-addition of an organolithium or a Grignard reagent to a *N*-*tert*-butanesulfinimine with high diastereoselectivity, followed by an acidic methanolysis of the sulfinamide product affording the corresponding amine in high yield. **4.2** represents the ideal chiral auxiliary serving as an activating group for nucleophilic addition to the imine,<sup>451</sup> preventing imine hydrolysis or competitive  $\alpha$ -deprotonation, it gives clean reactions and it is easy to remove.<sup>452</sup> In fact, **4.2** is a superior chiral auxiliary compared to the *p*-toluenesulfinamide group reported by Davis;<sup>453</sup> the former has a lower molecular weight, reacts much easier with aldehydes and ketones and provides an enhanced diastereofacial selectivity.<sup>451,452</sup>

Asymmetric synthesis of levocetirizine was previously performed either by resolution,<sup>454,455</sup> with stoichiometric heavy metal chiral oxazaborolidine reduction<sup>456</sup> or by preparative chiral HPLC separation.<sup>457</sup> Sepracor<sup>446</sup> and AstraZeneca<sup>110,458</sup> have recently reported the use of the Ellman-Davis sulfinamide chemistry for the formal synthesis of levocetirizine through diastereoselective synthesis of the amine intermediate (*R*)-**4.3** (see Scheme 4.2.1).



**Scheme 4.2.1:** Formal asymmetric synthesis of (*R*)-levocetirizine via diastereoselective organolithium addition to a *N*-*tert*-butanesulfinimine affording the (*R*)-enantiomer of the amine intermediate **4.3**.<sup>458</sup>

Interestingly, it was demonstrated that (*S*)-**4.3** was achieved with Grignard reagents through a six-membered chelating transition state, whereas organolithium reagents afforded the (*R*)-**4.3** through a non-chelating addition (see Figure 4.2.1).<sup>458</sup> Sepracor later reported an improved diastereoselective synthesis (93:7 *dr*) of (*R*)-**4.3** in 80% yield using triisopropylbenzene sulfonamide instead of *tert*-butanesulfonamide.<sup>110</sup>



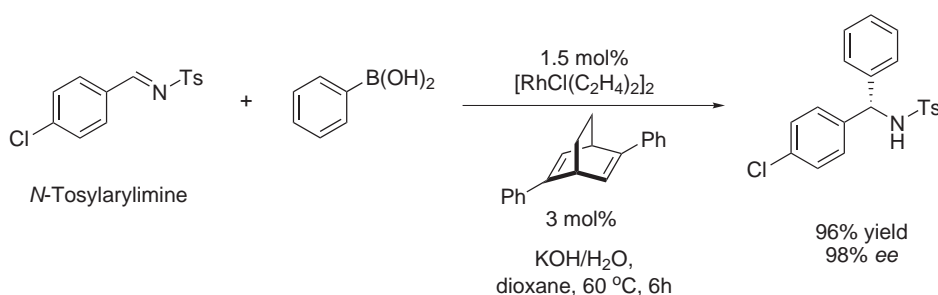
**Figure 4.2.1:** Choice of organometallic reagent determines the diastereo-facial attack; Grignard reagents afford the (*S*)-configuration through a six-membered chelating transition state, whereas organolithium reagents afford the (*R*)-configuration through a non-chelating addition.<sup>458</sup>  $R^1 = 4\text{-ClC}_6\text{H}_4$ ,  $R^2 = \text{C}_6\text{H}_5$

## 4.2.2 Catalytic enantioselective syntheses

Even though the Ellman-Davis method has many advantages, the use of organolithium or Grignard reagents is subject to some concerns. These highly reactive species require inconvenient reaction conditions (low temperature and dry solvents) and they are incompatible with many functional groups such as nitro, ketone and amine functionalities. The use of arylboronic acids has therefore been investigated, because this group of reagents represents a unique class of organometallic compounds. Arylboronic acids are stable compounds and many of them are commercially available with a variety of different functionalities (more than 1000 different arylboronic acids are commercially available).<sup>459</sup> Although arylboronic acids are poor nucleophiles, rhodium(I) complexes has been found to catalyze their addition to *N*-phenylsulfonimines as reported in 2000 by Miyaura and co-workers.<sup>460</sup> High functional group tolerance was demonstrated by related rhodium-catalyzed additions of arylboronic acids to aldehydes and  $\alpha,\beta$ -unsaturated

ketones.<sup>461,462</sup>

In 2004 asymmetric methods were independently published by the Tomioka<sup>463</sup> and Hayashi<sup>464</sup> groups, inspired by early work on enantioselective addition of arylstannanes.<sup>465</sup> Tomioka and co-workers<sup>463</sup> obtained only modest enantioselectivity with 3 mol% Rh(acac)(C<sub>2</sub>H<sub>4</sub>)<sub>2</sub> and a variety of amidophosphane ligands (see Figure 4.2.2) applied to *N*-tosylarylimines. On the other hand, Hayashi and co-workers<sup>464</sup> obtained both high yield and high enantioselectivity with 1.5 mol% [RhCl(C<sub>2</sub>H<sub>4</sub>)<sub>2</sub>]<sub>2</sub> and the chiral diene (*R,R*)-Ph-bod\* using similar substrates (see Scheme 4.2.2 for an example).

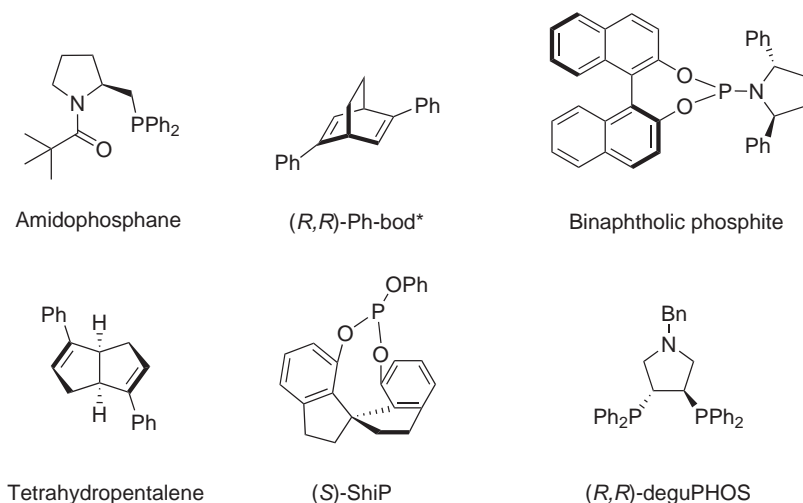


**Scheme 4.2.2:** Enantioselective addition of an arylboronic acid to a *N*-tosylarylimine. Many similar examples were reported by Hayashi and co-workers giving high yield and enantioselectivity.<sup>464</sup>

A number of other chiral ligands has been reported in the literature for this type of chemistry, such as binaphtholic phosphites,<sup>466</sup> tetrahydropentalenes,<sup>467</sup> (*S*)-ShiP<sup>468</sup> and (*R,R*)-deguPHOS<sup>469</sup> (see Figure 4.2.2).

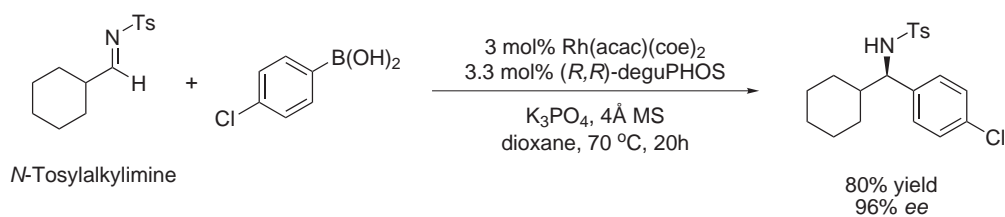
One major limitation of these methods is the removal of the *N*-tosyl group, which is difficult and requires harsh conditions, typically using large excess of Sml<sub>2</sub>.<sup>463</sup> These conditions severely limit the presence of some functional groups, and in fact protodehalogenation has been observed by the use of Sml<sub>2</sub>/HMPA,<sup>464</sup> which would be rather inconvenient, e.g. for the industrial synthesis of levocetirizine. The Hayashi group improved this limitation by replacing the *N*-tosyl group with *N*-nosyl which was successfully cleaved by treatment with PhSH and K<sub>2</sub>CO<sub>3</sub>.<sup>470</sup>

Another major limitation, especially noted by Hayashi, is that these additions are limited to arylimines.<sup>464</sup> This was however solved by the Ellman group. The enantioselective addition to *N*-tosylalkylimines or *N*-diphenylphosphinoimines could be catalyzed by Rh(acac)(coe)<sub>2</sub> in the presence of a chiral ligand, (*R,R*)-deguPHOS, (see Scheme 4.2.3).<sup>471</sup> In this study from 2008 a variety of chiral ligands was tested, among others



**Figure 4.2.2:** Chiral ligands widely used in rhodium-catalyzed enantioselective additions of arylboronic acids to *N*-sulfonimines.<sup>463,464,466–469</sup>

the tetrahydropentalene and (*S*)-ShiP illustrated in Figure 4.2.2, but (*R,R*)-deguPHOS was found to be the best.<sup>471</sup> Fortunately, this ligand is commercially available and does not require a lengthy synthesis as some of the other chiral ligands, such as (*R,R*)-Ph-bod\*. A diastereoselective version has also been reported using dppbenz as ligand with *N*-*tert*-butanesulfinalkylimines<sup>469</sup> and *N*-*tert*-butanesulfinimino esters.<sup>472</sup>



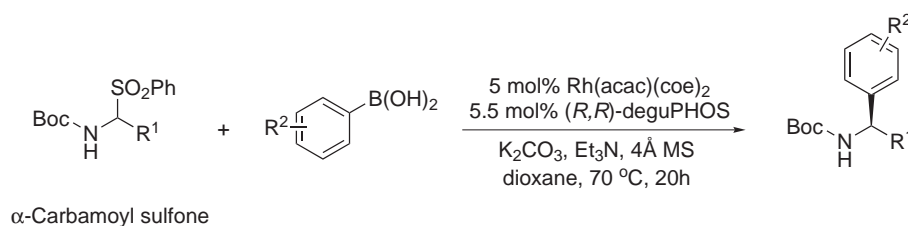
**Scheme 4.2.3:** Enantioselective synthesis of an  $\alpha$ -aryl alkylamine using Rh(acac)(coe)<sub>2</sub> and (*R,R*)-deguPHOS. Many examples are reported by the Ellman group.<sup>471</sup>

### 4.2.3 Diaryl amines from $\alpha$ -carbamoyl sulfones

Concurrently, the Ellman group also worked on improving the enantioselective synthesis of diaryl amines using the same catalytic conditions; Rh(acac)(coe)<sub>2</sub> and (*R,R*)-

deguPHOS (see Table 4.2.1).<sup>473</sup> Besides of these changes, the imine substrate was also changed. Activated imines are very reactive and difficult to manipulate and purify,<sup>474</sup> but Ellman and co-workers found, that  $\alpha$ -carbamoyl sulfones could be used as *N*-Boc protected imine precursors,<sup>473</sup> inspired by previous reports using this approach for imine preparation.<sup>475–477</sup>

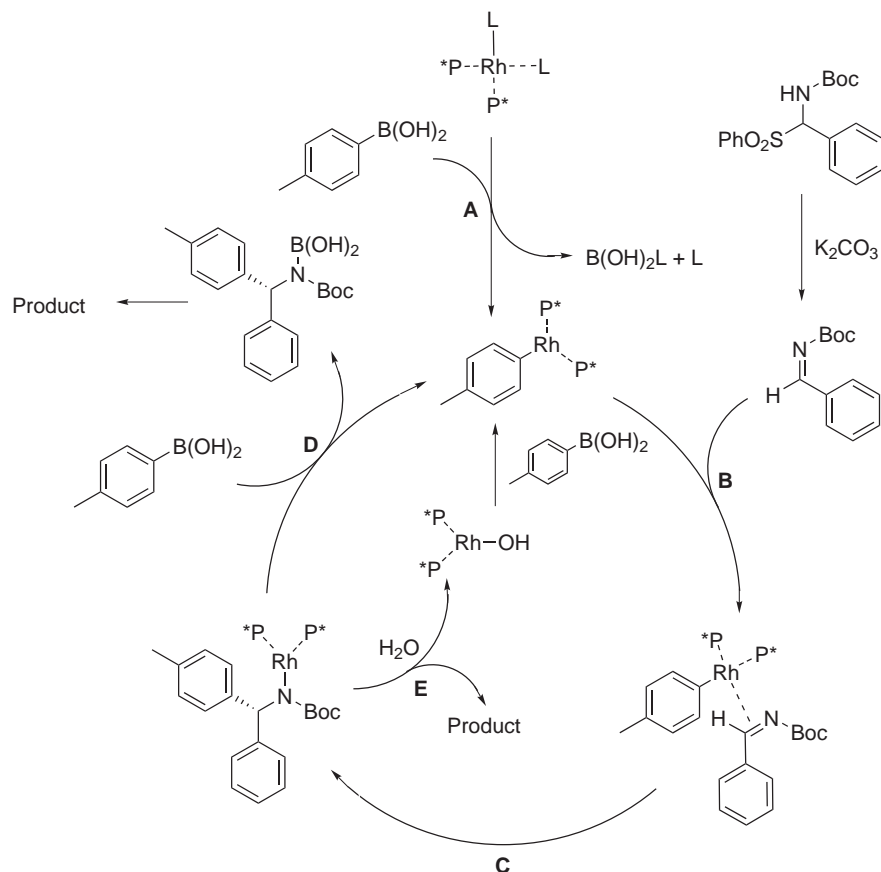
**Table 4.2.1:** Synthesis of various *N*-Boc diaryl amines.<sup>473</sup>



| entry | $R^1$   | $R^2$             | yield (%) <sup>a</sup> | ee (%) <sup>b</sup> |
|-------|---|-------------------|------------------------|---------------------|
| 1     | Ph  | 4-Cl              | 76                     | 98 <sup>c</sup>     |
| 2     | Ph  | 4-Me              | 70                     | 96                  |
| 3     | Ph  | 4-MeO             | 76                     | 93 <sup>c</sup>     |
| 4     | Ph  | 4-CF <sub>3</sub> | 51                     | 95 <sup>c</sup>     |
| 5     | Ph  | 3-Cl              | 55                     | 99                  |
| 6     | Ph  | 3-Me              | 66                     | 95                  |
| 7     | Ph  | 3-Ac              | 52                     | 94                  |
| 8     | Ph  | 2-Me              | 62                     | 93                  |
| 9     | 4-MeC <sub>6</sub> H <sub>4</sub>               | H                 | 71                     | 90                  |
| 10    | 3-MeC <sub>6</sub> H <sub>4</sub>               | H                 | 70                     | 95                  |
| 11    | 2-MeC <sub>6</sub> H <sub>4</sub>               | H                 | 63                     | 97                  |
| 12    | 4-BrC <sub>6</sub> H <sub>4</sub>               | H                 | 59                     | 90                  |
| 13    | 2-thienyl <sup>d</sup>                          | H                 | 71                     | 96                  |
| 14    | 4-MeOC <sub>6</sub> H <sub>4</sub>              | H                 | 76                     | 96 <sup>c</sup>     |
| 15    | 4-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub> | H                 | 69                     | 79 <sup>c</sup>     |

<sup>a</sup>: Isolated yields after chromatography. <sup>b</sup>: Enantiomeric purity determined by chiral HPLC analysis. <sup>c</sup>: Absolute configuration established by comparison of the optical rotation of amine obtained upon Boc cleavage to literature values.<sup>458,465</sup>

*N*-Boc imines are considerably hydrolytic labile, but the corresponding  $\alpha$ -carbamoyl sulfones are stable and solid compounds generating the imine *in situ* upon treatment with base. The use of *N*-Boc protection is furthermore superior compared to e.g. the *N*-tosyl group, because of the low molecular weight, easy cleavage conditions (typically TFA or HCl) and simple removal of deprotection by-products due to their volatility.<sup>478</sup> These improvements make the synthesis of the free amine much more straightforward.



**Figure 4.2.3:** Proposed mechanism for the rhodium-catalyzed enantioselective addition of boronic acid to in situ generated arylimine.<sup>479</sup>

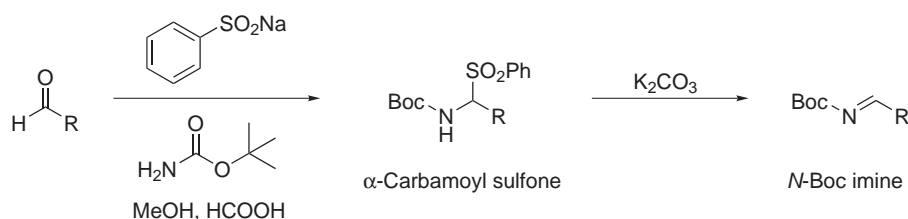
As illustrated in Table 4.2.1 these optimized reaction conditions gave good yields and high enantioselectivities for almost all substrates. The chemistry demonstrates both compatibility with heterocyclic imines and with *ortho*-substituted derivatives. Dioxane was found to be the best solvent with regard to yield and enantioselectivity.<sup>473</sup> Unfortunately, full conversion was never achieved, which might be a result of competitive substrate hydrolysis<sup>473</sup> or more likely by decomposition of the  $Rh(I)$ -aryl complex resulting in formation of an arene.<sup>480</sup>

In analogy to mechanisms proposed in the literature,<sup>479,481–483</sup> a reasonable mechanism can be established for this chemistry (see Figure 4.2.3). Initially, a transmetalation between the arylboronic acid and the rhodium catalyst takes place forming a  $Rh(I)$ -aryl complex (**A**). Rhodium hereby gets a free coordination site which is believed to

coordinate the imine (**B**). Through an insertion, the aryl-group is then added to the imine affording a catalyst product species (**C**). The final step in the catalytical cycle is the regeneration of the Rh(I)-aryl complex through a transmetalation with a new arylboronic acid (**D**). The desired product is formed by protonation of the boron species. Alternatively, the product is formed directly by protonation from water, originating from the arylboronic acid (**E**), regenerating the Rh(I)-aryl complex.<sup>479</sup>

#### 4.2.3.1 Utilization of $\alpha$ -carbamoyl sulfones as imine precursors

$\alpha$ -Carbamoyl sulfones are easily synthesized from the corresponding aldehyde, *tert*-butyl carbamate and benzenesulfinic acid sodium salt in the presence of formic acid.<sup>476</sup> Upon treatment with base the *N*-Boc imine is formed (see Scheme 4.2.4).



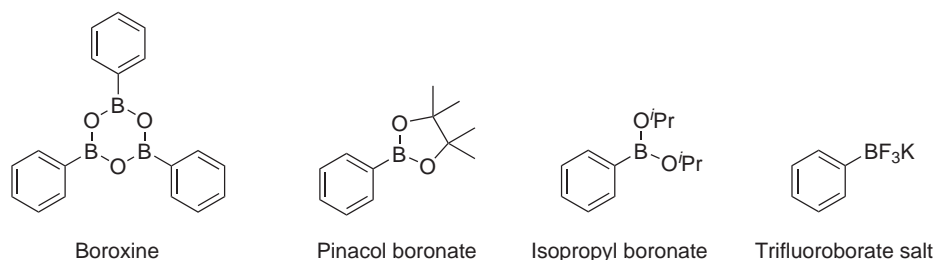
**Scheme 4.2.4:** Synthesis of  $\alpha$ -carbamoyl sulfones from the corresponding aldehyde and subsequent in situ formation of the *N*-Boc imine upon treatment with an inorganic base.<sup>473</sup>

Ellman and co-workers<sup>473</sup> found that addition of both  $\text{K}_2\text{CO}_3$  (6 equiv) and  $\text{Et}_3\text{N}$  (1.5 equiv) was necessary to improve the yield of the  $\alpha$ -aryl arylmethylaniline (see Table 4.2.1).  $\text{K}_2\text{CO}_3$  is believed to act as base for generation of the imine, but the action of  $\text{Et}_3\text{N}$  is rather unclear. In the absence of  $\text{Et}_3\text{N}$ , the yield dropped 20%. Since  $\text{K}_2\text{CO}_3$  is not fully soluble in dioxane,  $\text{Et}_3\text{N}$  might neutralize the acidic boronic acid to prevent decomposition of the Rh(I)-aryl complex.<sup>480</sup>

#### 4.2.3.2 Arylboronic acid versus boroxine as coupling partner

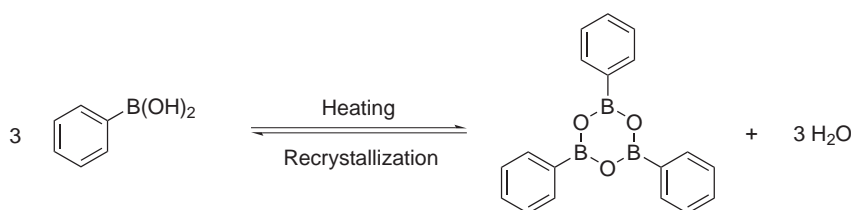
With respect to the arylboron reagent, Batey and co-workers<sup>484</sup> demonstrated that arylboronic acid gave the highest yield and stereoselectivity in similar chemistry compared to other boronic acid equivalents such as phenylpinacol boronate ester, phenylisopropyl boronate ester and phenyl potassium trifluoroborate salt (see Figure 4.2.4).

In most reports it is not stated explicitly whether the boronic acid or the corresponding boroxine was used, despite the fact, that commercially available arylboronic acids



**Figure 4.2.4:** Examples of different arylboron reagents tested in the rhodium-catalyzed addition to imines.<sup>463,484</sup>

contain varying amounts of boroxine (see Figure 4.2.5).<sup>251</sup> However, Tomioka and co-workers<sup>463</sup> found that one of their major improvements was using the arylboroxine instead of the arylboronic acid. This was not true for the rhodium-catalyzed addition to *in situ* generated *N*-Boc imines reported by the Ellman group;<sup>473</sup> the arylboronic acids were recrystallized from water to decompose any present boroxine.<sup>485,486</sup>



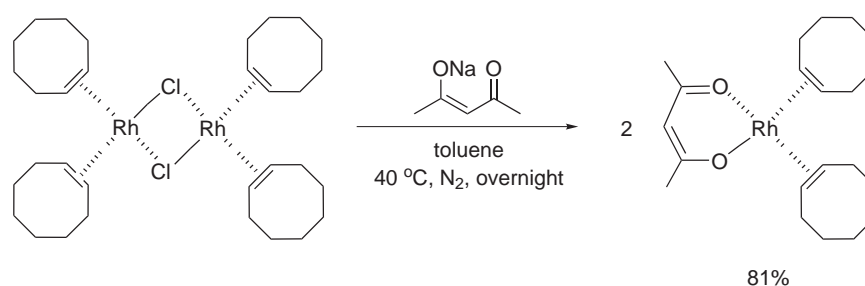
**Figure 4.2.5:** Arylboronic acids can exist both as a single boronic acid and as a boroxine, which is a cyclic anhydride of the corresponding boronic acid.<sup>487</sup>

Arylboroxines have been known for a long time, and it is well known that the ease of formation is dependent on the substituents, e.g. boroxine formation is favored by *ortho*-hydroxy substituents<sup>488</sup> and electron-donating groups.<sup>487</sup> Heating converts boronic acids into the corresponding boroxine by elimination of water.<sup>489</sup> On the other hand, hydrolysis of boroxines readily proceeds in the presence of water<sup>485,486</sup> and is accelerated by electron-withdrawing groups.<sup>487</sup> The equilibrium between the arylboronic acids and the corresponding boroxine was found to be reversible at room temperature and driven by entropic forces in solution.<sup>487</sup>



### 4.3 Aim of project

Despite the many optimizations, this chemistry still suffers from a number of drawbacks. The optimal catalyst,  $\text{Rh}(\text{acac})(\text{coe})_2$ , is currently not commercially available and has to be synthesized from  $[\text{RhCl}(\text{coe})_2]_2$  and  $\text{Na}(\text{acac})$  under a strictly inert atmosphere, preferably in a nitrogen-filled glovebox (see Scheme 4.3.1).<sup>490</sup>  $\text{Rh}(\text{acac})(\text{coe})_2$  is highly air-sensitive required that the reaction is set up in a glovebox and run in small sealed vials. This is inconvenient for most research laboratories; it is tedious and scaling-up is difficult. Moreover, the preparation of dry and degassed solvents and reagents is time-consuming.



**Scheme 4.3.1:** Synthesis of  $\text{Rh}(\text{acac})(\text{coe})_2$  from  $[\text{RhCl}(\text{coe})_2]_2$ .<sup>490</sup>

It was therefore desirable to further optimize the reaction with regard to the catalyst and to develop a practically more convenient procedure compared to the previous results from the Ellman group (c.f. Table 4.2.1).<sup>473</sup> The aim of the project is outlined below:

- Implementation of a commercially available Rh-catalyst
- Catalyst is preferably air-stable
- Good yield and excellent enantioselectivity
- Dependency of boroxine should be investigated
- Usage of glovebox should be omitted
- A functionalized diaryl amine should be scaled up to gram-scale

In addition to the implementation of a commercially available Rh-catalyst, dependency of boroxine should also be investigated. So far, no general procedure for determination of boroxine in the arylboronic acid batch does exist. And the actual dependency of

## Aim of project

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boroxine on this particular reaction still remains unexplored eventhough it might be important. Finally, the optimized reaction should be utilized for a gram-scale synthesis of a functionalized diaryl amine to demonstrate the usefulness of the chemistry.

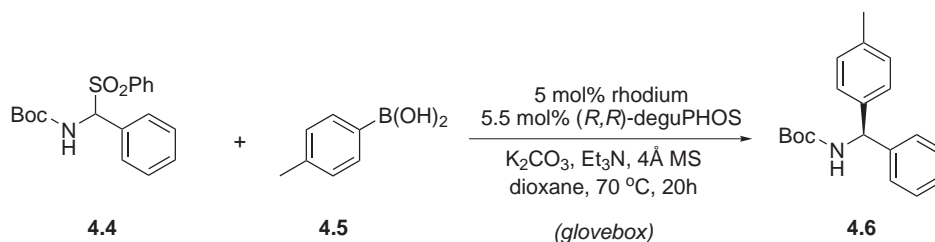
## 4.4 Results and discussion

### 4.4.1 Optimization of model reaction

#### 4.4.1.1 Initial screening of rhodium catalysts

Based on the many reports on enantioselective addition of arylboronic acids to imines, it seems that  $[\text{RhCl}(\text{C}_2\text{H}_4)_2]_2$ <sup>464,467,470,471</sup> and  $\text{Rh}(\text{acac})(\text{C}_2\text{H}_4)_2$ <sup>463,466,468,479</sup> are the most predominant catalysts. Presumably, the explanation for this observation is the profound lability of the ethylene ligand ensuring fast ligand exchange with the chiral ligand. Other rhodium catalysts with less labile ligands have also been reported, such as  $[\text{RhCl}(\text{cod})]_2$ <sup>463</sup> and the cationic catalyst  $[\text{Rh}(\text{cod})(\text{MeCN})]\text{BF}_4$ .<sup>460,484</sup> However, the latter has been found to be an ineffective catalyst in several reactions, either because of low yield<sup>469,472</sup> or low enantioselectivity.<sup>468</sup>

**Table 4.4.1:** Screening of commercially available Rh-catalysts.



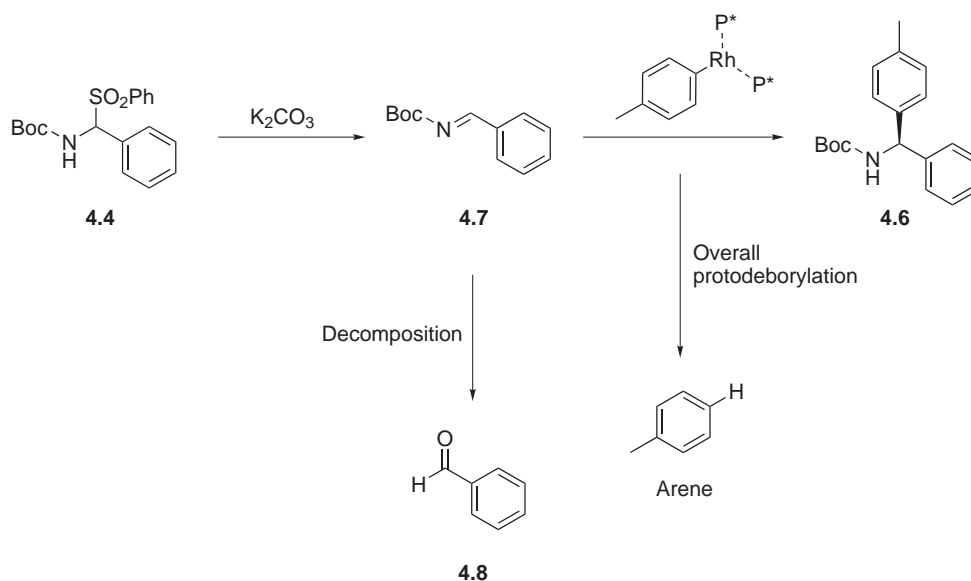
| entry | catalyst   | price (\$) <sup>a</sup> | yield (%) <sup>b</sup> | ee (%) <sup>c</sup> |
|-------|--|-------------------------|------------------------|---------------------|
| 1     | $[\text{RhCl}(\text{coe})_2]_2$                  | 1.50                    | 47                     | 95                  |
| 2     | $\text{Rh}(\text{acac})(\text{C}_2\text{H}_4)_2$ | 1.28                    | 72                     | 92                  |
| 3     | $[\text{RhCl}(\text{C}_2\text{H}_4)_2]_2$        | 0.84                    | 38                     | 90                  |
| 4     | $\text{Rh}(\text{acac})(\text{cod})$             | 1.30                    | 53                     | 85                  |
| 5     | $[\text{RhCl}(\text{cod})]_2$                    | 0.50                    | 63                     | 40                  |
| 6     | $[\text{Rh}(\text{OH})(\text{cod})]_2$           | 0.62                    | 87                     | 24                  |
| 7     | $\text{Rh}(\text{acac})(\text{coe})_2$           | — <sup>d</sup>          | 70                     | 96                  |

<sup>a</sup>: Estimated price per reaction based on the cheapest available supplier. <sup>b</sup>: Purified by flash chromatography. <sup>c</sup>: Determined by chiral HPLC. <sup>d</sup>: Catalyst is not commercially available.

Inspired by these reports, a broad range of commercially available neutral rhodium catalysts were screened with (*R,R*)-deguPHOS as chiral ligand in a model reaction forming the diaryl amine **4.6** from the  $\alpha$ -carbamoyl sulfone **4.4** and 4-methylphenylboronic acid (**4.5**) (see Table 4.4.1). The reaction conditions reported by Ellman and co-workers<sup>473</sup> in 2007 were chosen. These conditions gave 70% yield and 96% ee with the original

catalyst,  $\text{Rh}(\text{acac})(\text{coe})_2$ , and the presented substrates **4.4** and **4.5** (entry 7). Synthesis of starting materials for the screening experiments are described in section 4.4.1.3.

$[\text{RhCl}(\text{coe})_2]_2$ ,  $\text{Rh}(\text{acac})(\text{C}_2\text{H}_4)_2$  and  $[\text{RhCl}(\text{C}_2\text{H}_4)_2]_2$  were the only catalysts giving comparable or higher enantioselectivities than the original catalyst (entry 1–3).<sup>473</sup> This corresponds to the enhanced lability of the *coe*- and ethylene-ligands. However, only  $\text{Rh}(\text{acac})(\text{C}_2\text{H}_4)_2$  gave similar yield to  $\text{Rh}(\text{acac})(\text{coe})_2$  (entry 2), whereas the two other catalysts gave much lower yields (entries 1 and 3).  $\text{Rh}(\text{acac})(\text{cod})$  gave modest yield with acceptable enantiomeric excess; 53% yield, 85% ee (entry 4). Unfortunately, none of these rhodium catalysts are significantly more stable than  $\text{Rh}(\text{acac})(\text{coe})_2$  and optimization would be difficult due to their air-sensitivity. Contrarily, good to excellent yield was achieved with  $[\text{RhCl}(\text{cod})]_2$  and  $[\text{Rh}(\text{OH})(\text{cod})]_2$  (entries 5–6), but not surprisingly, the enantioselectivities were very low. On the other hand, these two catalysts were the cheapest commercially rhodium catalysts available (see Table 4.4.1). The cyclooctadiene (*cod*) ligand is tightly bound to rhodium and ligand exchange with (*R,R*)-*deguPHOS* only occurs slowly. Apparently, the rhodium catalyst itself catalyzes the racemic addition to the imine, resulting in a low stereoselectivity.



**Scheme 4.4.1:** *Destiny of the starting material **4.4**, which is converted to the imine **4.7** in situ. If the rhodium catalyst does not work, decomposition leading to the aldehyde **4.8** takes place, otherwise the desired cross-coupling yields the diaryl methyl amine **4.6**.*

Analyzing these initial screenings revealed that full conversion was never achieved, not

[Rh(OH)(cod)]<sub>2</sub> and [RhCl(cod)]<sub>2</sub> are the two most desirable catalysts based on their enhanced air-stability and low price. Both catalysts gave good to excellent yield in the initial screening experiments, but very low enantioselectivities. To increase the apparent ligand exchange with (*R,R*)-deguPHOS, preincubation of catalyst and ligand prior to addition of starting materials was tested (see Table 4.4.2). Preincubation was introduced previously in the Ellman group for improvement of enantioselectivity.<sup>471</sup> Two different methods were used; catalyst/ligand-only and all-at-once preincubation. The former is a preincubation of catalyst and ligand only, followed by addition of starting materials, bases, molecular sieves and the latter is a preincubation with bases and molecular sieves present.

4.4 + 4.5  $\xrightarrow[\text{K}_2\text{CO}_3, \text{Et}_3\text{N}, 4\text{\AA MS, dioxane, } 70\text{ }^\circ\text{C, } 20\text{h}]{\text{5 mol\% rhodium, 5.5 mol\% (R,R)-deguPHOS, preincubated 1h, } 70\text{ }^\circ\text{C}}$  4.6

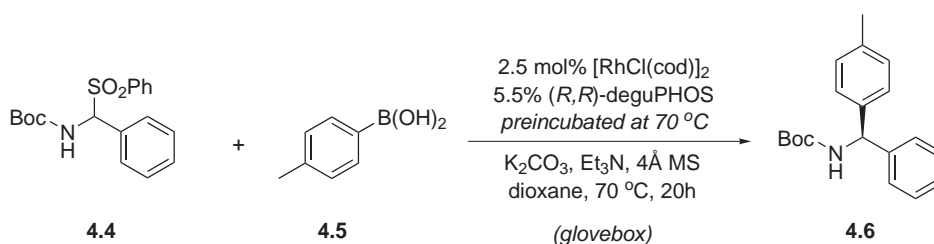
*(glovebox)*

<sup>a</sup>: Purified by flash chromatography. <sup>b</sup>: Determined by chiral HPLC. <sup>c</sup>: Preincubation of catalyst and ligand only for 1 hour at 70 °C, followed by addition of starting materials, bases and MS. <sup>d</sup>: Preincubation of catalyst and ligand with bases and molecular sieves for 1 hour at 70 °C, followed by addition of starting materials.

It was gratifying to discover that preincubation indeed had a huge impact on the enantioselectivity when  $[\text{RhCl}(\text{cod})]_2$  was used. Catalyst/ligand-only preincubation gave up to 96% ee (entry 3), whereas the all-at-once method gave a slightly lower selectivity, namely 91% ee (entry 4). This is a major improvement compared to the method with no preincubation, which gave a selectivity of only 40% ee (see Table 4.4.1, entry 6). On the other hand, preincubation experiments with  $[\text{Rh}(\text{OH})(\text{cod})]_2$  did not increase the enantioselectivity at all (see Table 4.4.2, entries 1–2). In fact, the selectivity was found to be somewhat reduced when preincubation was employed with this catalyst.

To further explore the impact of preincubation, a set of experiments with  $[\text{RhCl}(\text{cod})]_2$  was set up (see Table 4.4.3). The two preincubation methods were tested against different times, to investigate the impact of time on enantioselectivity.

**Table 4.4.3:** Time of preincubation of  $[\text{RhCl}(\text{cod})]_2$  and (*R,R*)-deguPHOS.



| entry | method                       | time (min) | yield (%) <sup>a</sup> | ee (%) <sup>b</sup> |
|-------|------------------------------|------------|------------------------|---------------------|
| 1     | Cat/ligand-only <sup>c</sup> | 15         | 63                     | 82                  |
| 2     | Cat/ligand-only <sup>c</sup> | 60         | 75                     | 96                  |
| 3     | Cat/ligand-only <sup>c</sup> | 120        | 71                     | 89                  |
| 4     | All-at-once <sup>d</sup>     | 15         | 78                     | 85                  |
| 5     | All-at-once <sup>d</sup>     | 60         | 85                     | 91                  |
| 6     | All-at-once <sup>d</sup>     | 120        | 64                     | 92                  |

<sup>a</sup>: Purified by flash chromatography. <sup>b</sup>: Determined by chiral HPLC. <sup>c</sup>: Preincubation of catalyst and ligand only for 1 hour at 70 °C, followed by addition of starting materials, bases and MS. <sup>d</sup>: Preincubation of catalyst and ligand with bases and molecular sieves for 1 hour at 70 °C, followed by addition of starting materials.

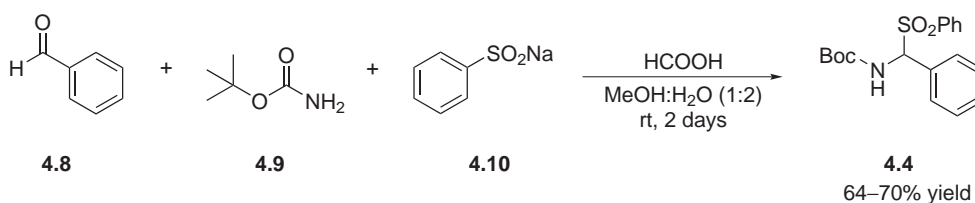
As shown in Table 4.4.3, the best enantioselectivity was obtained with the catalyst/ligand-only method at 70 °C for 1 hour (entry 2). Preincubation in just 15 minutes did also increase the selectivity (82% ee, entry 1), whereas preincubation in 2 hours lowered the enantioselectivity slightly to 89% ee (entry 3). These results indicate, that a too short preincubation does not ensure a sufficient ligand exchange with (*R,R*)-deguPHOS and a too long preincubation apparently slightly decomposes the reactive rhodium complex.

The results furthermore indicate, that the all-at-once method (entries 4–6) was not beneficial to the catalyst/ligand-only method.

Substitution of  $\text{Rh}(\text{acac})(\text{coe})_2$  with the commercially available and inexpensive  $[\text{RhCl}(\text{cod})]_2$  is of great importance. The latter catalyst is air-stable, commercially available and the cheapest one of those tested here.  $[\text{RhCl}(\text{cod})]_2$  is 2.5 times cheaper than  $\text{Rh}(\text{acac})(\text{C}_2\text{H}_4)_2$ .<sup>491</sup> Especially the enhanced catalyst stability makes the further optimization easier and development of a procedure outside the glovebox is more reasonable to succeed (see Section 4.4.2).

#### 4.4.1.3 Synthesis of $\alpha$ -carbamoyl sulfone

As mentioned in section 4.2.3.1  $\alpha$ -carbamoyl sulfones are easily synthesized from the corresponding aldehyde, *tert*-butyl carbamate (**4.9**) and benzenesulfinic acid sodium salt (**4.10**) in the presence of formic acid. Thus, the  $\alpha$ -carbamoyl sulfone **4.4** was synthesized from benzaldehyde (**4.8**) in 64–70% yield according to a literature procedure, which reported a 80% yield (see Scheme 4.4.2).<sup>476</sup> The synthesis employs cheap starting materials and no purification step is required because the product **4.4** precipitates from the solvent mixture. This makes the synthesis convenient and nevertheless efficient and easy to scale up in gram-scale.



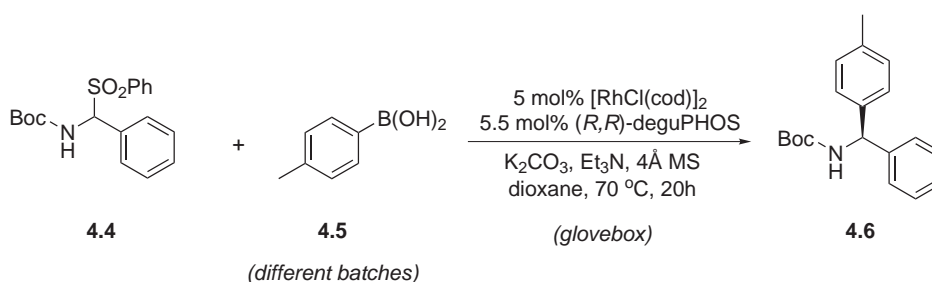
**Scheme 4.4.2:** Synthesis of the  $\alpha$ -carbamoyl sulfone **4.4** in 64–70% yield according to a literature procedure.<sup>476</sup>

#### 4.4.1.4 Investigation of the dependency of boroxine and water

According to the results obtained by Ellman and co-workers,<sup>473</sup> boronic acids were recrystallized from water prior to use.<sup>485,486</sup> The content of boroxine is primarily dependent on the drying procedure, because drying of boronic acids are known to form the corresponding boroxine.<sup>489</sup> The batch of boronic acid used so far contained only traces of boroxine determined by  $^1\text{H}$  NMR in dry  $\text{DMSO}-d_6$ . However, the exact influence of boroxine on this chemistry has not yet been investigated. Four different batches of

4-methylphenylboronic acid (**4.5**) were therefore prepared, using different drying conditions, and tested in the model reaction (see Scheme 4.4.4).

**Table 4.4.4:** Investigation of the dependency of boroxine and water.



| entry | drying                    | boroxine (%) <sup>a</sup> | water (%) <sup>a</sup> | conv. (%) <sup>b</sup> |
|-------|---------------------------|---------------------------|------------------------|------------------------|
| 1     | Not recryst. <sup>c</sup> | 17                        | 19                     | 40                     |
| 2     | — <sup>d</sup>            | <5                        | 80                     | 56                     |
| 3     | 2h (rt) <sup>e</sup>      | <5                        | 22                     | 74                     |
| 4     | 1h (100 °C) <sup>f</sup>  | 84                        | <5                     | 25                     |

<sup>a</sup>: Composition determined by <sup>1</sup>H NMR in dry DMSO-*d*<sub>6</sub> based on integrals. <sup>b</sup>: Conversion determined by <sup>1</sup>H NMR integrals compared to 1,3,5-trimethoxybenzene as internal reference. <sup>c</sup>: Used directly from the bottle without recrystallization. <sup>d</sup>: Recrystallized from H<sub>2</sub>O but not dried to remove excess water. <sup>e</sup>: Recrystallized from H<sub>2</sub>O and dried in high vacuum at room temperature. <sup>f</sup>: Recrystallized from H<sub>2</sub>O and dried in high vacuum at 100 °C.

In the first experiment **4.5** was used directly from the bottle and thus not recrystallized from water (entry 1). This resulted in a reduced conversion (40%), which might be a result of either the content of boroxine (17%) or water (19%). Then a non-dried batch of recrystallized **4.5** was tested giving modest results (entry 2); 56% conversion was achieved which indicate that a high content of water (80%) did not reduce the yield significantly. Apparently, the molecular sieves are efficient enough to avoid hydrolysis of the *in situ* generated imine. Drying of the recrystallized batch of **4.5** for 2 hours at room temperature removed most of the water (22% remains) and only traces of boroxine was formed (<5%). This batch gave superior results, 74% conversion. Finally a batch of **4.5** containing 84% boroxine and only traces of water was prepared by drying at 100 °C for an hour. This batch gave only 25% conversion which clearly indicated, that boroxine is indeed undesired. Unfortunately, preparation of boronic acids in this way is highly dependent on initial water content and drying capacity of the vacuum pump. Therefore sequential drying is recommended using <sup>1</sup>H NMR in dry DMSO-*d*<sub>6</sub> to analyze the batch regularly during the drying process. Boronic acids should not contain more than 5% boroxine and approximately 20–30% water (cf. entry 3).

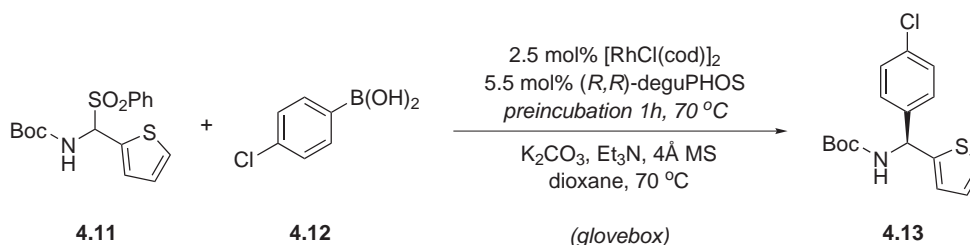


## 4.4.2 Synthesis of functionalized diaryl amine

### 4.4.2.1 Small-scale glovebox procedure

With the optimized reaction conditions in hand, a more functionalized diaryl amine was chosen for further optimization and up-scaling. This was done to demonstrate the usefulness of the chemistry. The Boc-protected (4-chlorophenyl)-(thiophen-2-yl)methylamine **4.13** was chosen as target molecule (see Table 4.4.5). **4.13** was synthesized identically with the optimized procedure described previously from 2-thiophenyl  $\alpha$ -carbamoyl sulfone **4.11** (see section 4.4.2.2) and from recrystallized 4-chlorophenylboronic acid (**4.12**).

**Table 4.4.5:** Initial synthesis of target molecule performed in the glovebox.



| entry | time (h) | conv. (%) <sup>a</sup> |
|-------|----------|------------------------|
| 1     | 1        | 25                     |
| 2     | 5        | 42                     |
| 3     | 10       | 67                     |
| 4     | 20       | 65                     |
| 5     | 40       | 66                     |

<sup>a</sup> Conversion determined by <sup>1</sup>H NMR using 1,3,5-trimethoxybenzene as internal standard.

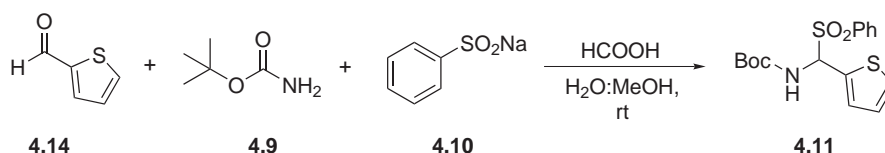
An investigation of the reaction time was conducted, since this parameter has not yet been considered. As shown in Table 4.4.5 a maximized conversion was achieved after 10 hours at 70 °C (entry 3). Interestingly, the conversion was neither increased nor decreased after approximately 10 hours (entries 3–5).

### 4.4.2.2 Optimization of 2-thiophenyl $\alpha$ -carbamoyl sulfone synthesis

Synthesis of the 2-thiophenyl  $\alpha$ -carbamoyl sulfone **4.11** was initially performed using the same procedure as for the synthesis of **4.4** (c.f. Scheme 4.4.2). Thiophene-2-carbaldehyde (**4.14**) was used as the starting material but only 30% yield was achieved

at room temperature (see Table 4.4.6, entry 1). This is significantly lower than for the synthesis of **4.4** which gave up to 70% isolated yield.

**Table 4.4.6:** Optimization of the 2-thiophenyl  $\alpha$ -carbamoyl sulfone synthesis.



| entry | H <sub>2</sub> O:MeOH <sup>a</sup> | time (days) | yield (%) <sup>b</sup> |
|-------|------------------------------------|-------------|------------------------|
| 1     | 2:1                                | 3           | 30                     |
| 2     | 2:1                                | 5           | 29                     |
| 3     | 5:1                                | 3           | 55                     |
| 4     | 7:1                                | 3           | 57                     |
| 5     | 10:1                               | 3           | 60                     |
| 6     | 1:0                                | 1           | 44                     |
| 7     | 1:0                                | 3           | 66                     |
| 8     | 1:0                                | 5           | 74                     |

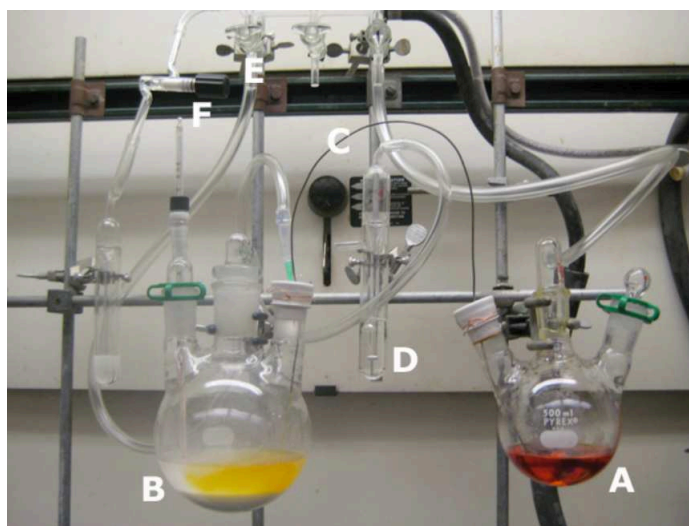
<sup>a</sup> Solvent mixture is given in volume ratio. <sup>b</sup> Isolated yield after trituration with H<sub>2</sub>O and Et<sub>2</sub>O.

An attempt to increase the yield of **4.11**, the reaction time was prolonged to 5 days, but that did not have any impact on the isolated yield (entry 2). Increasing the temperature from room temperature to 50 °C was unfavored; no product was isolated at all. This was probably due to the thermo instability of the product.

Another approach for improving the yield of **4.11** would be to decrease its solubility in the solvent mixture. A series of experiments using an increasing amount of water (entry 3–5) improved the yield significantly and 60% yield of the  $\alpha$ -carbamoyl sulfone **4.11** was isolated after three days using H<sub>2</sub>O:MeOH (10:1) (entry 5). Ultimately, the reaction was run in pure water (entry 6–8), and the isolated yield was slightly increased to 66% after three days (entry 7). Extending the reaction time further increased the yield to 74% after a total of 5 days (entry 8). Besides of these optimizations, reducing the solvent volume was also tested, but with no improvement of the isolated yield. Increasing the equivs of the aldehyde **4.14** from 1.5 to 2.5 equivalents gave only a slightly improvement of the yield. On large scale (40 mmol), **4.11** was obtained in 60–75% isolated yield as a white solid after a reaction time of three days utilizing the optimized reaction conditions.

#### 4.4.2.3 Large-scale procedure using cannulation technique

At first, the optimized reaction conditions were applied to the synthesis of the functionalized diaryl amine **4.13** on small scale (0.25 mmol) (see Table 4.4.7). Utilization of Schlenk flasks, reaction bombs and sealed vials outside the glovebox were tested, but all of them resulted in severe yield reduction giving only 14–39% conversion. The main problem was the preincubation, because the reaction flask had to be opened for addition of starting materials, bases and molecular sieves. Even though a nitrogen atmosphere was maintained carefully during preincubation, during addition of starting materials and during the actual reaction, oxygen apparently still got into the flasks and deactivated the reactive rhodium catalyst.

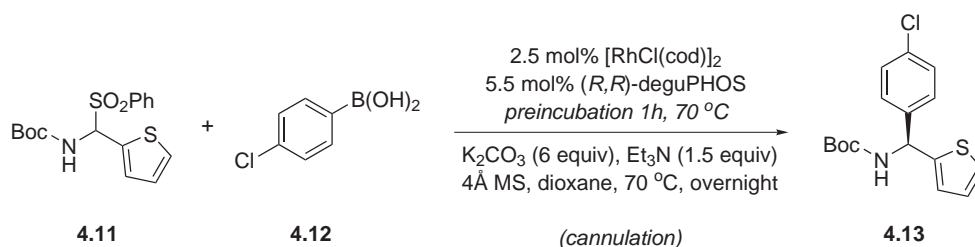


**Figure 4.4.1:** Cannulation technique for transferring the preincubation catalyst/ligand to the starting materials, bases and molecular sieves. **A:** catalyst/ligand solution, **B:** starting materials, bases and molecular sieves, **C:** the cannula, **D:** gas bubbler for receiving flask, **E:** nitrogen-inlet for receiving flask which must be closed in order to start the cannulation, **F:** valve to close the overall outlet to force the nitrogen flow through the cannula.

To solve this problem, it was apparent that a complete inert atmosphere had to be applied and maintained throughout the entire procedure. Furthermore, the chosen technique should be easy to scale up. It was therefore evident to use a cannulation technique for transferring the preincubated rhodium catalyst and ligand mixture to a solution of the starting materials without exposure to air (see Figure 4.4.1). Application of regular round-bottomed flasks has several advantages; first of all they are readily available, secondly up-scaling is easy and finally they make it possible to evacuate and back-fill with

nitrogen several times to establish the sufficient inert atmosphere.

**Table 4.4.7:** *Synthesis and up-scaling of functionalized amine.*



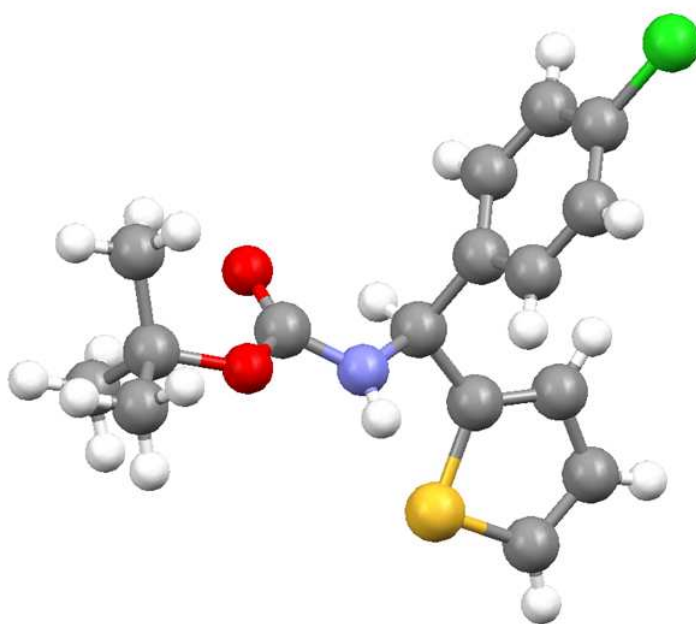
| entry | scale (mmol) | yield (%) <sup>a</sup> | ee (%) <sup>b</sup> |
|-------|--------------|------------------------|---------------------|
| 1     | 0.25         | 65                     | 99                  |
| 2     | 10           | 57                     | 95                  |
| 3     | 20           | 59                     | 95                  |
| 4     | 20           | 65                     | 95                  |

<sup>a</sup>: Purified by chromatography. <sup>b</sup>: Determined by chiral HPLC.

With the implementation of the cannulation technique, synthesis of the target molecule **4.13** was performed on several scales up to 20 mmol (7.07 g) (see Table 4.4.7, entries 3–4). The large scale synthesis gave a 59–65% purified yield and an enantioselectivity of 95% ee.

#### 4.4.2.4 Determination of absolute configuration

So far, the absolute configuration of the diaryl amines **4.6** and **4.13**, and all the products reported by Ellman and co-workers<sup>473</sup> were established based on comparison of literature values of optical rotations of a few amines obtained after Boc cleavage (cf. Table 4.2.1). Crystals were grown of **4.13** from a saturated solution in EtOH using slow evaporation to determine the absolute configuration. The absolute configuration was shown by anomalous dispersion to be (*S*) using X-ray crystallography (see Figure 4.4.2), which confirms the previous tentative assignment unequivocally. (*R,R*)-deguPHOS apparently provides the same stereofacial addition which is reasonable when the same stereoisomer of the ligand is used. Determination of the absolute configuration of **4.13** was performed in cooperation with graduate student MaryAnn Robak and the X-ray Diffraction Facility at UC Berkeley, College of Chemistry.



**Figure 4.4.2:** Absolute configuration of **4.13** was shown to be (*S*) using X-ray crystallography. The crystals were grown from a saturated solution in EtOH by slow evaporation.

## 4.5 Conclusion

A series of neutral rhodium catalysts were screened in a model reaction forming the diaryl amine **4.6**. The synthesis employed an enantioselective addition of 4-methylphenylboronic acid (**4.5**) to an *in situ* generated imine originated from the  $\alpha$ -carbamoyl sulfone **4.4**. It was found that  $[\text{RhCl}(\text{cod})]_2$  gave similar yields to the original catalyst,  $\text{Rh}(\text{acac})(\text{coe})_2$ , but with poor enantioselectivity. This is presumably a result of insufficient ligand exchange with the chiral ligand (*R,R*)-deguPHOS. Preincubation was therefore introduced by stirring the catalyst and ligand for 1 hour at 70 °C prior to addition of starting materials, bases ( $\text{K}_2\text{CO}_3$  and  $\text{Et}_3\text{N}$ ) and molecular sieves. An enantioselectivity of 95–99% ee was hereby obtained with a purified yield of **4.6** typically in the range of 63–75%. The utilization of  $[\text{Rh}(\text{OH})(\text{cod})]_2$  was also tested, but it was not possible to obtain enantioselectivities above 24% ee with this catalyst, even with preincubation. The influence of boroxine present in the boronic acid batch was carefully investigated. It was evident that a substantial amount of boroxine (84%) had an unfavorable effect on the yield of **4.6** giving conversions as low as 25%. Actually, only small amounts of boroxine (17%) gave a reduced conversion (down to 40%). On the other hand, water did not have the same impact on the outcome. A wet batch of the boronic acid **4.5** (80% water) did only reduce the conversion to 56% (compared to 74% which was the best result). In conclusion, it was found that boronic acids should not contain more than 5% boroxine and approximately 20–30% water. Finally, the optimized reaction conditions were tested for the synthesis of the highly functionalized diaryl amine **4.13** from 2-thiophenyl  $\alpha$ -carbamoyl sulfone **5.11** and 4-chlorophenylboronic acid (**4.12**). Yields in the range of 57–67% and enantioselectivities up to 99% ee were obtained by carrying out the synthesis in a glovebox. Synthesis of **4.11** was optimized by changing the solvent mixture from  $\text{H}_2\text{O}:\text{MeOH}$  (2:1) to pure  $\text{H}_2\text{O}$  which resulted in a significant increase of yield going from 30% to 66% after 3 days. The synthesis of **4.13** was scaled up 80 times to a 20 mmol scale (7.07 g) and performed outside the glovebox by using a cannulation technique to transfer the preincubated catalyst and ligand giving up to 65% purified yield and 95% ee. Absolute configuration was determined to be (*S*) using X-ray crystallography. Through substitution of the rhodium catalyst, introduction of a preincubation step, analysis of the boroxine influence and utilization of a cannulation technique, the enantioselective addition of boronic acids to *in situ* generated imines has been optimized. Now, it represents a very useful method for the synthesis of diaryl amines using commercially available starting materials only and with no use of a glovebox. Moreover, it is easy to scale up in practically any quantity, and it was demonstrated that yield and enantioselectivity were retained at large scale.

## 4.6 Experimental

### General procedures

The chemicals were all commercially available and used without further purification unless otherwise stated. Solvents were of HPLC quality. 1,4-Dioxane was passed through a column of dry, activated, basic alumina under a N<sub>2</sub> atmosphere. The solvent was transferred to the reaction flask via a syringe without exposure to air. 4-Methylphenylboronic acid (**4.5**) and 4-chlorophenylboronic acid (**4.12**) were recrystallized from H<sub>2</sub>O prior to use (see below for details). DMSO-*d*<sub>6</sub> was dried over 4 Å 3.2 mm pellets molecular sieves prior to use. Powdered molecular sieves (4 Å, <5 microns) were activated under high vacuum at 230–260 °C overnight. Et<sub>3</sub>N was freshly distilled over CaH<sub>2</sub> under a N<sub>2</sub> atmosphere. If Et<sub>3</sub>N had to be used in the glovebox, it was degassed using three consecutive freeze-pump-thaw cycles. Evaporation *in vacuo* was performed on a rotary evaporator at approx. 40 °C down to approx. 20 mbar. TLC was performed using Dynamic Adsorbents, Inc. glass plates coated with 250 μm F<sub>254</sub> silica gel. 15 vol% EtOAc in hexanes was used as the eluent and the plates were visualized by UV (Spectroline<sup>®</sup>, Model EF-140C, short wave UV 254 nm) and subsequently stained with PMA (10 g phosphomolybdic acid + 100 mL absolute EtOH). Unless otherwise stated, air-sensitive reactions were run in a VAC Omni-lab glovebox filled with dry nitrogen. Flash chromatography was performed using Merck KGaA silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM) according to a general procedure reported by Still.<sup>492</sup> <sup>1</sup>H NMR (300 MHz) and <sup>13</sup>C NMR (75 MHz) spectra were recorded on a Bruker Avance AV-300 and <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra on a Bruker AVB-400 using deuterated solvents as internal references. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. Melting points were measured on a Mel-Temp<sup>®</sup> 3.0 from Laboratory Devices, Inc. USA, and they are given in degree Celsius (°C) uncorrected. MS was determined on a HPLC/MS system from Agilent and IR was measured on a FT-IR spectrometer using attenuated total reflection (ATR) sampling technique. Enantiomeric excess was determined by chiral HPLC using an Agilent 1100 series instrument and a Chiralpak<sup>®</sup> AS-H column (amylose tris[(*S*)-α-methylbenzyl-carbamate] coated on a 5 μm silica gel), L = 250 mm, I.D. = 4.6 mm, from Danicel Technologies, LTD. 4 vol% EtOH in hexanes was used for analyzing **4.6**, whereas 1% EtOH in hexanes was used for analyzing **4.13**. Both protocols were run isocratic with a flow rate of 1.00 mL/min at max. 70 bar for 25 minutes. For both compounds, a racemate synthesized with dppbenz was used to determine the retention times of each enantiomer and secure sufficient baseline separation. Elemental analysis and X-ray crystallography were obtained from in-house facilities at College of Chemistry, University of California at Berkeley.

**tert-Butyl phenyl(phenylsulfonyl)methylcarbamate (4.4)**<sup>476</sup>

*tert*-Butyl carbamate (**4.9**) (5.00 g, 42.68 mmol, 1.0 equiv) and benzenesulfinic acid sodium salt (**4.10**) (14.00 g, 85.28 mmol, 2.0 equiv) were suspended in MeOH (40 mL) and H<sub>2</sub>O (80 mL). Benzaldehyde (**4.8**) (6.5 mL, 6.80 g, 64.08 mmol, 1.5 equiv) and 95% HCOOH (3.2 mL, 3.80 g, 82.55 mmol, 2.0 equiv) were added. A clear, colorless solution appeared. The flask was loosely closed with a glass stopper and the reaction mixture was stirred vigorously at room temperature for 2 days. During that time a white precipitate formed. The suspension was filtered and the crude product was triturated with H<sub>2</sub>O (2 × 20 mL) and Et<sub>2</sub>O (2 × 20 mL). Dried overnight in high vacuum afforded pure **4.4** as a white solid (9.40–10.30 g, 64–70%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.92 (d, *J* = 7.6 Hz, 2H), 7.66–7.63 (m, 1H), 7.56–7.52 (m, 2H), 7.43 (m, 5H), 5.93 (d, *J* = 10.6 Hz, 1H), 5.76 (d, *J* = 10.1 Hz, 1H), 1.26 (s, 9H). The spectral values were in accordance with the literature.<sup>476</sup> Mp 180–181 °C (lit.:<sup>476</sup> N/A °C).

**Recrystallization of 4-methylphenylboronic acid (4.5)**

In a conical flask was added **4.5** (10 g) and H<sub>2</sub>O (400 mL) and the flask was loosely closed with a watch glass. The suspension was heated to boiling over the course of 25 minutes on a heating plate (115 °C) under vigorous stirring. Boiling was maintained for 5 minutes to fully dissolve the boronic acid. The hot solution was filtered through a filter paper using gravity filtration to remove insoluble particles. The colorless solution was cooled to ambient temperature overnight and then cooled in an ice bath for 1 hour. The crystallized boronic acid **4.5** and was isolated by vacuum filtration and dried by continuing the vacuum filtration for additional 15 minutes. To remove further amounts of water the boronic acid was dried in high vacuum at room temperature until <sup>1</sup>H NMR in dry DMSO-*d*<sub>6</sub> showed a composition of no more than 5% boroxine and approx. 20–30% water, which was equivalent to 8.0–8.6 g of white microplates. **4.5** exhibits the following chemical shifts: <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.92 (s, broad, 2H), 7.68 (d, *J* = 7.2 Hz, 2H), 7.13 (d, *J* = 7.5, 2H), while the corresponding boroxine exhibits these shifts:  $\delta$  7.77 (d, *J* = 7.5 Hz, 2H), 7.18 (d, *J* = 7.4 Hz, 2H).

**(R)-tert-Butyl phenyl(4-methylphenyl)methylcarbamate (4.6)**

In a nitrogen-filled glovebox, [RhCl(cod)]<sub>2</sub> (3.1 mg, 6.25  $\mu$ mol, 0.025 equiv) was suspended in dry dioxane (1 mL) in a small oven-dried vial. (*R,R*)-deguPHOS (7.4 mg, 13.75  $\mu$ mol, 0.055 equiv) was added and the vial was capped and stirred for 1 hour at 70 °C outside the glovebox. Then, the vial was brought back to the glovebox and opened. Upon preincubation the catalyst completely dissolved and the resultant mixture was a clear, dark orange solution. Dioxane (3 mL) was added followed by the  $\alpha$ -carbamoyl sulfone **4.4** (86.0 mg, 0.25 mmol, 1.0 equiv) and recrystallized 4-methylphenylboronic acid (**4.5**) (67.9 mg, 0.50 mmol, 2.0 equiv). Finally, dry K<sub>2</sub>CO<sub>3</sub> (207 mg, 1.50 mmol, 6.0 equiv), dry and degassed Et<sub>3</sub>N (52  $\mu$ L, 38.0 mg, 0.38 mmol, 1.5 equiv) and activated, powdered 4 Å molecular sieves (400 mg) were added and the vial was sealed and



stirred vigorously overnight (or as indicated) at 70 °C outside the glovebox. The crude product was cooled to ambient temperature and filtered through Celite<sup>®</sup>, which was washed with EtOAc (40 mL). The combined organic filtrates were evaporated to dryness *in vacuo* and subsequently purified by flash chromatography (5→15 vol% EtOAc in hexanes) affording **5.6** as a white solid (55.7 mg, 75%) with 96% ee determined by chiral HPLC; (*R*)-enantiomer (major): 10.5 minutes, (*S*)-enantiomer (minor): 12.6 minutes. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.33–7.23 (m, 5H), 7.12 (m, 4H), 5.88 (m, 1H), 5.15 (m, 1H), 2.31 (s, 3H), 1.43 (s, 9H). The spectral values were in accordance with the literature.<sup>473</sup> MS (ESI+) *m/z* 310 (M<sup>+</sup> + Na). Mp 123–126 °C (lit.:<sup>473</sup> N/A °C).

#### **tert-Butyl phenylsulfonyl(thiophen-2-yl)methylcarbamate (4.11)**

In a round-bottomed flask benzenesulfinic acid sodium salt (**4.10**) (13.13 g, 80.00 mmol, 2.0 equiv) was dissolved in H<sub>2</sub>O (105 mL). *tert*-Butyl carbamate (**4.9**) (4.69 g, 40.00 mmol, 1.0 equiv) was added, but did not dissolve. 2-Thiophene-carboxaldehyde (**4.14**) (5.50 mL, 6.73 g, 60.00 mmol, 1.5 equiv) was added forming a yellow emulsion. 95% HCOOH (3.10 mL, 3.68 g, 80.00 mmol, 2.0 equiv) was added. The flask was loosely fitted with a glass stopper and the white, opaque, biphasic mixture was stirred vigorously at room temperature. After a couple of hours the water phase became clear. The product **4.11** was formed as yellow chunks, which became more dispersed in the water phase as the reaction proceeded. After 3 days of stirring the suspension was filtered. The yellow chunks were crushed with a spatula, and the product was triturated with H<sub>2</sub>O (2 × 10 mL) and Et<sub>2</sub>O (2 × 10 mL). After each trituration the solvent was removed by vacuum filtration. Finally, it was dried for an hour under high vacuum to give the α-carbamoyl sulfone **3.13** as a white solid (9.34 g, 66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.93 (d, *J* = 7.6 Hz, 2H), 7.65 (t, *J* = 7.2 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 2H), 7.42 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.27 (d, *J* = 3.4 Hz, 1H), 7.07 (dd, *J* = 4.9, 3.7 Hz, 1H), 6.19 (d, *J* = 10.8 Hz, 1H), 5.68 (d, *J* = 10.5 Hz, 1H), 1.26 (s, 9H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 153.3, 136.5, 134.2, 131.5, 129.6, 129.4, 129.2, 127.8, 127.4, 81.5, 70.3, 28.1. Elem. anal. calcd. for C<sub>16</sub>H<sub>19</sub>NO<sub>4</sub>S<sub>2</sub>: C, 54.37; H, 5.42; N, 3.96; found: C, 54.00; H, 5.68; N, 4.00. Mp 162–164 °C (decomp.). IR (neat) *ν* 3347, 2955, 1699, 1511, 1306, 1144 cm<sup>-1</sup>.

#### **Recrystallization of 4-chlorophenylboronic acid (4.12)**

4-Chlorophenylboronic acid (**4.12**) was recrystallized analogously to **4.5**. It exhibits the following chemical shifts: <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 8.16 (s, broad, 2H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.39 (d, *J* = 8.3, 2H), while the corresponding boroxine exhibits these shifts: δ 7.86 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 2H).

**(S)-tert Butyl (4-chlorophenyl)(thiophen-2-yl)methylcarbamate (4.13)**

An oven-dried three-necked round-bottomed flask with a magnetic stir bar was equipped with a vacuum adaptor in the middle neck and glass stoppers in the two other necks. The adaptor was connected to a Schlenk line and the flask was cooled to ambient temperature under high vacuum. The flask was carefully back-filled with nitrogen and then charged with  $[\text{RhCl}(\text{cod})]_2$  (247 mg, 0.50 mmol, 0.025 equiv) and (*R,R*)-deguPHOS (583 mg, 1.10 mmol, 0.055 equiv) by removing one of the glass stoppers. A septum was used to seal the flask and the other glass stopper was exchanged with an adaptor equipped with a thermometer. The flask was then evacuated under high vacuum and carefully back-filled with nitrogen three times. A positive flow of nitrogen was thereafter maintained to ensure an oxygen-free atmosphere inside the flask. Dry dioxane (80 mL) was added through the septum via a syringe and the flask was submerged into an oil bath (70 °C), and the mixture was stirred for 1 h (internal temperature reached 65 °C after 20 min). Initially, the catalyst was not fully soluble in dioxane, but as the preincubation proceeded it completely dissolved. The solution of the active catalyst was clear and dark orange.

Meanwhile, an oven-dried, three-necked round-bottomed flask with a magnetic stir bar was equipped with a vacuum adaptor in the middle neck and glass stoppers in the two other necks. The adaptor was connected to a Schlenk line and the flask was cooled to ambient temperature under high vacuum. The flask was carefully back-filled with nitrogen and then charged with  $\alpha$ -carbamoyl sulfone **4.11** (7.07 g, 20.00 mmol, 1.0 equiv), recrystallized 4-chlorophenylboronic acid (**4.12**) (6.26 g, 40.00 mmol, 2.0 equiv), dry  $\text{K}_2\text{CO}_3$  (16.58 g, 120.00 mmol, 6.0 equiv) and activated, powdered 4Å molecular sieves (32 g) by removing one of the glass stoppers. A septum was used to seal the flask, and the other glass stopper was exchanged with an adaptor equipped with a thermometer. The flask was then evacuated under high vacuum and carefully back-filled with nitrogen three times. A positive nitrogen inflow was thereafter maintained to ensure an oxygen-free atmosphere inside the flask. Dry dioxane (240 mL) was added through the septum via a syringe immediately before the preincubation was complete (described above). Additionally, dry  $\text{Et}_3\text{N}$  (4.20 mL, 3.04 g, 30.00 mmol, 1.5 equiv) was added via a syringe. The white suspension was stirred vigorously at room temperature while adding the preincubated solution of catalyst and ligand via cannula transfer resulting in a yellow suspension. The reaction flask was submerged into an oil bath (70 °C), and the yellow suspension was stirred vigorously for 16 h (internal temperature: 70 °C). The yellow suspension was allowed to cool to ambient temperature over the course of one hour and vacuum filtered through Celite<sup>®</sup>, which was rinsed with EtOAc (300 mL). The combined yellow filtrates were evaporated to dryness *in vacuo* to give a yellow solid. The crude product was purified by flash chromatography using a gradient of 5→15 vol% EtOAc in hexanes which affording the title compound **5.13** as a white solid (3.82–4.21 g, 59–65%) with 95% ee determined by chiral HPLC; (*R*)-enantiomer (minor): 11.1 minutes, (*S*)-enantiomer (major): 13.5 minutes. The absolute configuration was determined by X-ray crystallography to be the (*S*)-enantiomer. Crystals were grown by preparing a saturated

solution of **4.13** in EtOH, which was filtered to remove insoluble particles. The filtrate was transferred to a scintillation glass and fitted loosely with a lid and allowed to slowly evaporate undisturbed at room temperature. The slow evaporation afforded needle-like crystals of **4.13** which were isolated by filtration.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33–7.25 (m, 5H), 7.23 (dd,  $J = 5.0, 1.1$  Hz, 1H), 6.92 (dd,  $J = 5.0, 3.6$  Hz, 1H), 6.78 (d,  $J = 3.7$  Hz, 1H), 6.08 (broad s, 1H), 5.26 (broad s, 1H), 1.43 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  154.6, 145.6, 140.3, 133.5, 128.7, 128.2, 126.8, 125.6, 125.3, 80.2, 70.1, 28.3. MS (ESI+)  $m/z$  346 ( $\text{M}^+ + \text{Na}$ , 100%), 347 (17%), 348 (40%). Elem. anal. calcd. for  $\text{C}_{16}\text{H}_{18}\text{ClNO}_2\text{S}$ : C, 59.34; H, 5.60; N, 4.33; found: C, 59.14; H, 5.58; N, 4.33. mp 138–140 °C. IR (neat)  $\nu$  3347, 2979, 2921, 2361, 1686, 1515, 1233, 1169  $\text{cm}^{-1}$ .  $[\alpha]_D^{20} +11.0$  ( $c = 0.5$ , EtOH).



# Chapter 5

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# Chapter 6

## Publications

### Included in this thesis:

*'Palladium-Catalyzed  $\alpha$ -Arylation of Tetramic Acids'*. Morten Storgaard, Florencio Z. Dörwald, Bernd Peschke, David Tanner *J. Org. Chem.* **2009**, *74*, 5032–5040.

*'Rhodium-Catalyzed Enantioselective Addition of Arylboronic Acids to in situ Generated N-Boc Arylimines. Preparation of (S)-tert-Butyl (4-chlorophenyl)(thiophen-2-yl)methylcarbamate'*. Morten Storgaard, Jonathan A. Ellman, Jason A. Bexrud, Mark Lautens *Org. Synth.*, **2009**, *86*, 360–373.

### Not included in this thesis:

*'Design, Synthesis and Biological Activity of Novel Reversible Peptidyl FVIIa Inhibitors'*. Morten Storgaard, Florencio Z. Dörwald, Bernd Peschke, David Tanner, *in preparation*

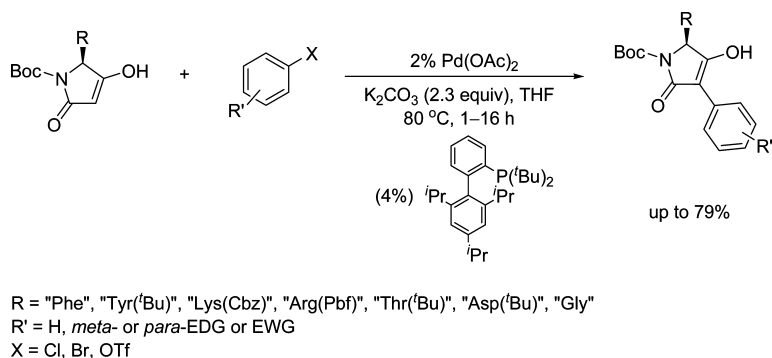
*'The Direct Acyl-Alkylation of Arynes. Preparation of Methyl 2-(2-acetylphenyl)acetate'*. David C. Ebner, Uttam K. Tambar, Brian M. Stoltz, Morten Storgaard, Nathan D. Ide, John A. Ragan, Jonathan A. Ellman *Org Synth.*, **2009**, *86*, 161–171.

Palladium-Catalyzed  $\alpha$ -Arylation of Tetramic AcidsMorten Storgaard,<sup>†,‡</sup> Florencio Zaragoza Dörwald,<sup>‡,§</sup> Bernd Peschke,<sup>‡</sup> and David Tanner<sup>\*,†</sup>

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Received April 17, 2009

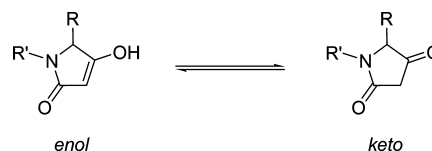


A mild, racemization-free, palladium-catalyzed  $\alpha$ -arylation of tetramic acids (2,4-pyrrolidinediones) has been developed. Various amino acid-derived tetramic acids were cleanly arylated by treatment with 2 mol % of Pd(OAc)<sub>2</sub>, 4 mol % of a sterically demanding biaryl phosphine, 2.3 equiv of K<sub>2</sub>CO<sub>3</sub> or K<sub>3</sub>PO<sub>4</sub>, and aryl chlorides, bromides, or triflates in THF. With conventional heating, conversions >95% could be attained after 1 h at 80 °C, whereas microwave-induced heating led to much shorter reaction times (5 min at 110 °C). The electron density of the aryl electrophile had no effect on their reactivity: both electron-rich and electron-poor aryl chlorides and bromides or triflates led to good yields. Ortho-substituted aryl halides and heteroaryl halides, however, did not undergo the title reaction.

## Introduction

Tetramic acids are  $\beta$ -keto- $\gamma$ -lactams which are slightly acidic ( $pK_a \approx 6.4$ ).<sup>1,2</sup> Depending on solvent, concentration, and temperature, tetramic acids can exist as both an enol (4-hydroxy-3-pyrrolin-2-one) and a keto tautomer (2,4-pyrrolidinedione) (see Figure 1).<sup>1,3,4</sup>

The structural unit of tetramic acids has been known for more than 100 years,<sup>5</sup> and it is found in many biologically active natural products,<sup>3</sup> typically either as 3-acyl or 4-*O*-alkyl derivatives, examples being althiomycin,<sup>6a,b</sup> dolastatin 15,<sup>6c,d</sup> and epicoccamide.<sup>6e</sup> Tetramic acids are important intermediates



**FIGURE 1.** Tetramic acids can exist as both an enol and a keto tautomer.<sup>1,3,4</sup>

in the synthesis of statins,<sup>7a,b</sup>  $\beta$ -hydroxy  $\gamma$ -amino acids,<sup>7c</sup> and lactams<sup>7d</sup> which are inhibitors of renin. Renin is involved in the renin–angiotensin system (blood pressure and fluid regulating system in the body), hypertension, congestive heart failure, and development of HIV. Furthermore, tetramic acid derivatives have been reported as key intermediates for the synthesis of analogues of penicillins and cephalosporins<sup>7e</sup> and 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives<sup>7f</sup> which are inhibitors of glycolic acid oxidase and thus potentially useful drugs for the treatment of calcium oxalate renal lithiasis (kidney stones) and primary hyperoxalurias, which is an inborn error of metabolism resulting in increased urinary excretion of oxalate. 2-Ethyl-4,6-dimethylphenyl-substituted tetramic acid derivatives have been described in the patent literature as novel pesticides

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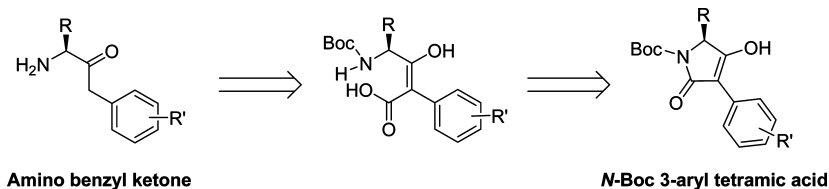
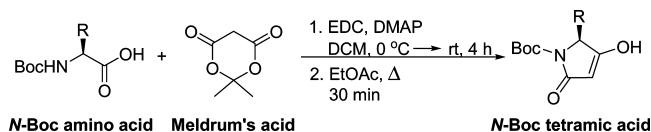
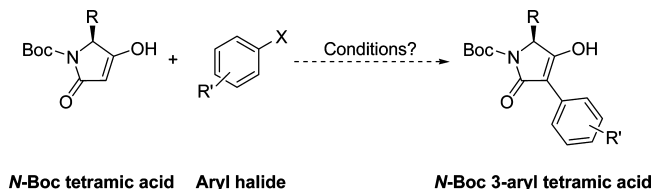


FIGURE 2. Retrosynthetic analysis of amino benzyl ketones.

SCHEME 1. General Synthesis of *N*-Boc-Protected Amino Acid-Derived Tetramic Acids<sup>8,9</sup>

and herbicides.<sup>7g</sup> Recently, methods for incorporation of amino acid-derived tetramic acids into peptides have been developed,<sup>8</sup> giving rise to more stable tripeptides. Tetramic acids derived from amino acids are easily synthesized in good yield from commercially available *N*-Boc amino acids and Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) via DCC (*N,N'*-dicyclohexylcarbodiimide) or EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) activation (see Scheme 1).<sup>8,9</sup>

Only a few examples in the literature have described the 3-aryl tetramic acids,<sup>10a,b</sup> the most important being the use of 3-phenyl 5-olefinic tetramic acids as novel glycine site *N*-methyl-D-aspartate receptor antagonists, for the treatment of neurological diseases.<sup>10c</sup> The development of a solid-phase synthesis of substituted 3-aryl tetramic acids has also been described.<sup>10d</sup> However, none of these methods utilize the easy synthesis of *N*-Boc protected tetramic acids described above, and the methods are not general, requiring the use of strong base (e.g., KHMDS or NaOEt) and many synthetic steps. None of these methods make it easy to efficiently synthesize a broad range of 3-aryl-substituted amino acid-derived tetramic acids as potentially interesting biologically active compounds. We therefore wished to develop a useful method for the synthesis of 3-aryl tetramic acids from the readily available *N*-Boc amino acid-derived tetramic acids.

SCHEME 2. Proposed  $\alpha$ -Arylation of Tetramic Acids with an Aryl Halide (X = Cl, Br, I)

Specifically, we want to use these compounds as building blocks for *C*-terminal modified peptides toward the preparation of peptidyl enzyme inhibitors. For example, ring-opening of the 3-aryl tetramic acids followed by decarboxylation would lead to a new type of amino benzyl ketones (see Figure 2), which subsequently can be coupled to the *C*-terminal of a peptide.

Traditionally,  $\alpha$ -arylated ketones or carboxylic acid derivatives have been synthesized by nucleophilic aromatic substitution reactions ( $S_NAr$ ) of aryls substituted with electron-withdrawing groups by reaction with stabilized enolates<sup>11</sup> or via copper-catalyzed enolate reaction with 2-bromobenzoic acid.<sup>12</sup> These methods all have drawbacks and are not very general. Usually, they require harsh reaction conditions, which are not suitable for protected, enantiomerically pure amino acid derivatives. Using a palladium-catalyzed  $\alpha$ -arylation would be much more efficient since these reactions are typically more general, mild, and broad in substrate scope.

The literature reports a number of palladium-catalyzed  $\alpha$ -arylation conditions for different substrates containing electron-withdrawing groups such as ketones,<sup>13a</sup> aldehydes,<sup>13b</sup> malonates,<sup>13c</sup> cyanoesters,<sup>13c</sup> sulfones,<sup>13d</sup> esters,<sup>13e</sup> amides,<sup>13f</sup> protected amino acids,<sup>13g</sup> piperidinones,<sup>13h</sup> and nitriles.<sup>13i</sup> Only a few examples of  $\alpha$ -arylation of 1,3-dicarbonyls have been described, and most of them are nonchiral and synthetically undemanding compounds. Most of the examples have been reported by Buchwald and co-workers,<sup>13a</sup> using substrates such as diethyl malonate, 1,3-cyclohexanedione, and 1,3-cyclopentanedione. Very recently more functionalized substrates have been subjected to palladium-catalyzed arylation, e.g. the  $sp^2$  arylation of azine *N*-oxides,<sup>14</sup>  $\alpha$ -arylation of highly function-

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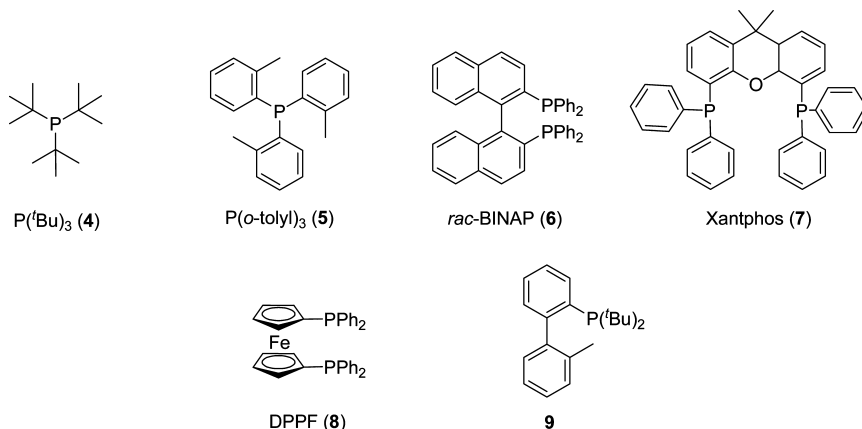


FIGURE 3. Ligands used in the initial screening experiments.

TABLE 1. Results from Initial Screening of Bases and Ligands

| entry | ligand           | base                            | conv (%) <sup>a</sup> |
|-------|------------------|---------------------------------|-----------------------|
| 1     | 4–8 <sup>b</sup> | all four <sup>b</sup>           | <5                    |
| 2     | 9                | Cs <sub>2</sub> CO <sub>3</sub> | 15                    |
| 3     | 9                | K <sub>3</sub> PO <sub>4</sub>  | 36                    |
| 4     | 9                | Na <sub>2</sub> CO <sub>3</sub> | — <sup>c</sup>        |
| 5     | 9                | K <sub>2</sub> CO <sub>3</sub>  | 36                    |

<sup>a</sup> Determined by <sup>1</sup>H NMR. <sup>b</sup> Ligands 4–8 tested with Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, and K<sub>2</sub>CO<sub>3</sub>, respectively, in 20 experiments. <sup>c</sup> No significant product formation determined by <sup>1</sup>H NMR or LC-MS.

alized cyclohexanones,<sup>15</sup> and asymmetric intramolecular  $\alpha$ -arylation of aldehydes.<sup>16</sup> However, to the best of our knowledge, tetramic acids have never before been subjected to this kind of transformation (see Scheme 2), and we set out to determine suitable reaction conditions.

Even though no general reaction conditions exist in the literature, it was possible to discern a general trend for the reaction of substrates similar to tetramic acids, e.g., 1,3-dicarbonyl compounds, cyclic substrates, and amino acids, from the literature. It was found that an  $\alpha$ -arylation is usually conducted with either Pd(OAc)<sub>2</sub> or Pd<sub>2</sub>(dba)<sub>3</sub> with use of an aryl bromide or iodide.<sup>13</sup> Aryl chlorides are often too unreactive for this type of chemistry. Many different solvents can be used, but THF, toluene, dioxane, MeCN, or DMF are the most common. The bases used can be divided into two groups: weak inorganic bases such as Cs<sub>2</sub>CO<sub>3</sub>, K<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>3</sub>PO<sub>4</sub>, or Na<sub>2</sub>CO<sub>3</sub> and strong organic bases such as NaOtBu, KHMDs, NaHMDs, LDA, or LiN(SiMe<sub>2</sub>Ph)<sub>2</sub>. The choice of base is strongly dependent on the pK<sub>a</sub> value of the substrate but, in general, strong bases can be used for most simple substrates. However, if base-sensitive functionalities are present in the molecule or deprotonation can cause racemization, strong bases may give problems. Sometimes strong bases even require a two-

TABLE 2. Increased Temperature and Prolonged Reaction Time

| entry | base                            | temp (°C) | time (h) | conv (%) <sup>a</sup> |
|-------|---------------------------------|-----------|----------|-----------------------|
| 1     | Cs <sub>2</sub> CO <sub>3</sub> | 80        | 72       | 21                    |
| 2     | K <sub>3</sub> PO <sub>4</sub>  | 80        | 72       | 33                    |
| 3     | Na <sub>2</sub> CO <sub>3</sub> | 80        | 72       | <5                    |
| 4     | K <sub>2</sub> CO <sub>3</sub>  | 80        | 72       | 32                    |
| 5     | Cs <sub>2</sub> CO <sub>3</sub> | 100       | 16       | 25                    |
| 6     | K <sub>3</sub> PO <sub>4</sub>  | 100       | 16       | 71                    |
| 7     | Na <sub>2</sub> CO <sub>3</sub> | 100       | 16       | <5                    |
| 8     | K <sub>2</sub> CO <sub>3</sub>  | 100       | 16       | 52                    |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

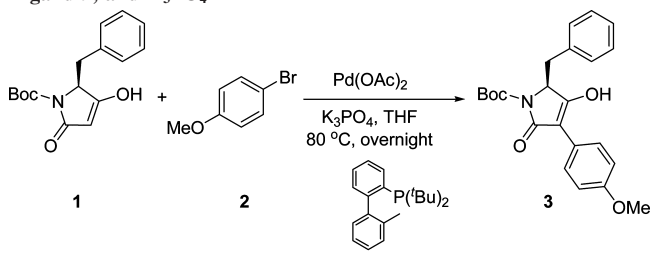
step procedure with addition of base at reduced temperature and then the actual arylation at elevated temperature.<sup>13h</sup> Buchwald and co-workers<sup>13a</sup> reported the first use of a weak inorganic base, K<sub>3</sub>PO<sub>4</sub>, in palladium-catalyzed  $\alpha$ -arylations of ketones. Finally, a very important parameter is the choice of ligand. This can be difficult, since many different ligands have been reported to work in  $\alpha$ -arylation of carbonyl substrates, with a broad variety of stereo- and electronic properties. In general the ligands are either mono- or bis-phosphines. With a rational selection of parameters it should be easier to find the optimal reaction conditions for the  $\alpha$ -arylation of tetramic acids (see Scheme 2).

## Results and Discussion

Initially, we tried to arylate Boc-pyPhe-OH (1) (the prefix “py” is used to indicate that the amino acid is converted to a tetramic acid<sup>8</sup>) with 4-bromoanisole (2) in the presence of 2 mol % of Pd(OAc)<sub>2</sub> in THF at 80 °C overnight for the synthesis of the 3-aryl tetramic acid 3 (see Table 1). Four different weak inorganic bases were chosen: Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, and K<sub>2</sub>CO<sub>3</sub>, respectively, in 2.3 equiv inspired by results published by Buchwald and co-workers.<sup>13a</sup> We prioritized the screening of a variety of weak bases because they are much more compatible with functional groups, and because tetramic acids

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**TABLE 3.** Variation of Equivalents of Aryl Bromide **2**, Pd(OAc)<sub>2</sub>, Ligand **9**, and K<sub>3</sub>PO<sub>4</sub>


| entry | Ar–Br <b>2</b><br>(equiv) | Pd(OAc) <sub>2</sub><br>(mol %) | ligand <b>9</b><br>(mol %) | K <sub>3</sub> PO <sub>4</sub><br>(equiv) | conv<br>(%) <sup>a</sup> |
|-------|---------------------------|---------------------------------|----------------------------|---|--------------------------|
| 1     | 2.0                       | 2                               | 4                          | 2.3                                       | 43                       |
| 2     | 1.0                       | 2                               | 4                          | 5.0                                       | 15                       |
| 3     | 1.0                       | 4                               | 8                          | 2.3                                       | >95                      |
| 4     | 1.0                       | 2                               | 8                          | 2.3                                       | >95                      |
| 5     | 1.0                       | 4                               | 4                          | 2.3                                       | 79                       |

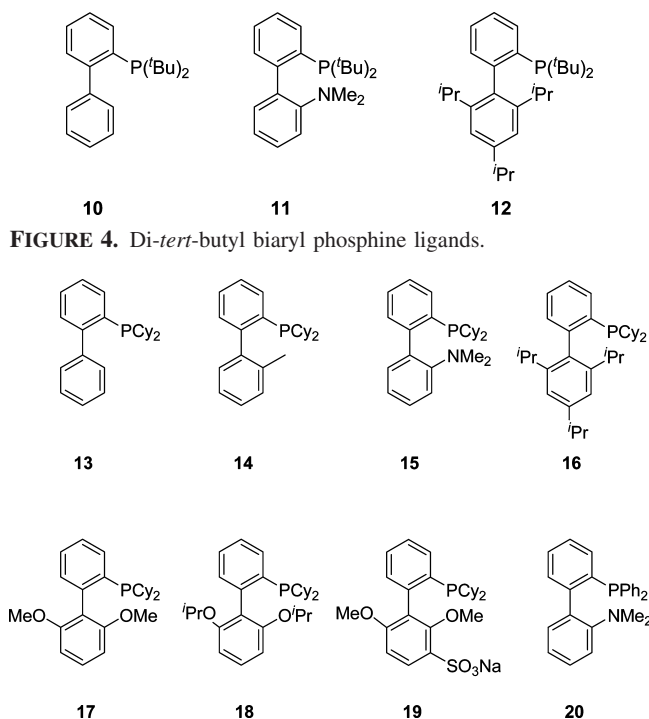
<sup>a</sup> Determined by <sup>1</sup>H NMR.

are slightly acidic. The phosphine ligands P(*t*-Bu)<sub>3</sub> (**4**), P(*o*-tolyl)<sub>3</sub> (**5**), *rac*-BINAP (**6**), Xantphos (**7**), and DPPF (**8**) (see Figure 3) were screened with the four bases in 20 initial experiments. Unfortunately, none of these conditions gave rise to any significant product formation (conv <5%) and only starting materials were isolated upon acidic workup (see Table 1, entry 1). However, to our gratification screening experiments with 4 mol % of biaryl phosphine ligand **9** gave promising results. Not surprisingly, a major difference among the bases was observed, K<sub>3</sub>PO<sub>4</sub> and K<sub>2</sub>CO<sub>3</sub> giving comparable results, 36% conversion (entries 3 and 5), whereas Na<sub>2</sub>CO<sub>3</sub> did not give any product formation at all (entry 4). Cs<sub>2</sub>CO<sub>3</sub> gave an intermediate result with a conversion of 15% (entry 2).

To further increase the conversion with 4 mol % of biaryl phosphine ligand **9** and 2.3 equiv of K<sub>3</sub>PO<sub>4</sub>, a series of experiments at elevated temperature (100 °C) and a series with prolonged reaction time (3 days) was conducted (see Table 2). No significant change in conversions was observed after 3 days (entries 1–4). On the other hand, the conversion was increased at 100 °C overnight, especially with K<sub>3</sub>PO<sub>4</sub>, which almost gave a 2-fold increase in conversion to 71% (entry 6). In both experimental series, the use of Na<sub>2</sub>CO<sub>3</sub> still did not give any significant product formation (entries 3 and 7).

We reasoned that inefficient activation of the catalyst may cause the low to moderate conversions obtained so far. Therefore we tested the screening reaction at 80 °C with all four bases, respectively, with 2 mol % of Pd<sub>2</sub>(dba)<sub>3</sub> as a direct source of Pd(0). However, we found that there was no improvement in conversion, and we therefore assumed that the problem with low conversion was not due to the nature of the palladium catalyst.

Having a set of reaction conditions giving a moderate conversion and a catalyst that presumably is sufficiently activated, we screened a set of different equivalents with regard to 4-bromoanisole (**2**), Pd(OAc)<sub>2</sub>, biaryl phosphine ligand **9**, and K<sub>3</sub>PO<sub>4</sub> (see Table 3). Increasing the equivalents of **2** from 1.0 to 2.0 only increased the conversion slightly (entry 1), whereas increasing the equivalents of K<sub>3</sub>PO<sub>4</sub> to 5.0 gave a significant reduction in conversion (entry 2). Furthermore, we examined the effect of catalyst and ligand loading. Increasing the loading of both catalyst and ligand to 4 and 8 mol %, respectively, gave full conversion of the starting material to the desired product **3**

**FIGURE 4.** Di-*tert*-butyl biaryl phosphine ligands.**FIGURE 5.** Dicyclohexyl (**13**–**19**) and diphenyl (**20**) biaryl phosphine ligands.

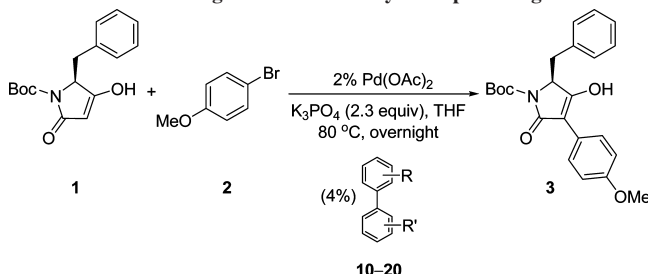
(entry 3). The same was true with increased ligand loading only (entry 4). It was found that an excess of ligand was essential, since 4 mol % of Pd(OAc)<sub>2</sub> and 4 mol % of biaryl phosphine ligand **9** gave a conversion of 79% (entry 5).

On the basis of these results, it seemed likely that screening other biaryl phosphine ligands might give full conversion without increased ligand loading. We therefore screened three classes of commercially available biaryl phosphine ligands: a series of di-*tert*-butyl biaryl phosphines **10**–**12** (see Figure 4), a series of dicyclohexyl biaryl phosphines **13**–**19**, and a single diphenyl biaryl phosphine **20** (see Figure 5). Only 2-di-*tert*-butylphosphino-2',4',6'-triisopropylbiphenyl (**12**) gave full conversion with 4 mol % of ligand loading (see Table 4, entry 3), the two other di-*tert*-butyl biaryl phosphines (**10** and **11**) gave only poor conversion (entries 1 and 2). All dicyclohexyl biaryl phosphine ligands **13**–**19** and the diphenyl biaryl phosphine ligand **20** gave only traces of product. Apparently, this reaction requires a sterically hindered and electron-rich ligand and the di-*tert*-butyl substituents are essential for reactivity, which is demonstrated by the absence of reactivity with the analogous dicyclohexyl biaryl phosphine ligand **16**.

Before moving on with substrate scope and limitations, we analyzed the enantiomeric purity of the product **3**, by means of chiral HPLC. Fortunately, little racemization had occurred (ee 97%), which was expected due to the use of mild base.

Having arrived at these optimized reaction conditions we wished to examine the scope and limitations of the reaction by testing other different aryl coupling partners, namely aryl chlorides, iodides, tosylates, and triflates. For comparison reasons we chose to screen 4-methoxy derivatives only (see Table 5). To our delight, 4-chloroanisole (**21**) reacted identically (entry 1) compared to 4-bromoanisole (**2**), as did aryl triflate **24** (entry 4). Use of aryl triflates expands the scope of the reaction further because it allows conversion of phenols into functional coupling partners very easily. Aryl iodide **22** and aryl tosylate **23** gave

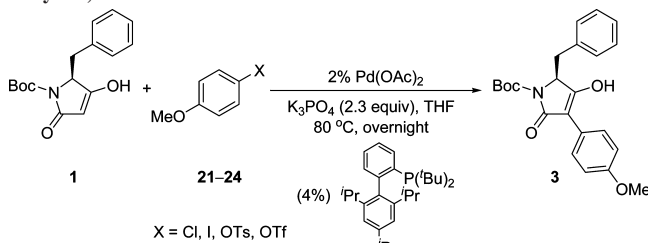
TABLE 4. Screening of Different Biaryl Phosphine Ligands



| entry | ligand       | conv (%) <sup>a</sup> |
|-------|--------------|-----------------------|
| 1     | <b>10</b>    | 5                     |
| 2     | <b>11</b>    | 21                    |
| 3     | <b>12</b>    | >95                   |
| 4     | <b>13–20</b> | <5                    |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

TABLE 5. Testing of Different Coupling Partners: Aryl Halides, Tosylate, and Triflate



| entry | Ar-X | conv. % <sup>a</sup> |
|-------|------|----------------------|
| 1     |      | >95                  |
| 2     |      | <5                   |
| 3     |      | 8                    |
| 4     |      | >95                  |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

only traces of the product **3** (entries 2 and 3). This observation is important because aryl chlorides are generally much cheaper than the corresponding iodides and a wider range of commercially available compounds exists.

To fine-tune the chemistry, reaction time and temperature were taken into consideration again. It was found that the reaction is in fact complete in less than an hour at 80 °C with 4-chloroanisole (**21**) as the coupling partner. The product is stable under the reaction conditions and the reaction time can be extended to 16–20 h with no product decomposition. In addition, lower temperatures were also tested. At room tem-

perature no reaction occurred and at 60 °C only 11% conversion was observed after 1 h. Therefore a temperature at 80 °C was chosen for further experiments.

With these suitable reaction conditions in hand, we once again examined the catalyst loading. Reducing the loading of Pd(OAc)<sub>2</sub> to 1 mol % and ligand **12** to 2 mol %, only 35% conversion was achieved after 1 h. However, full conversion was achieved with overnight reaction times. As previously shown, K<sub>3</sub>PO<sub>4</sub> and K<sub>2</sub>CO<sub>3</sub> gave similar conversions. We chose to use K<sub>2</sub>CO<sub>3</sub> exclusively because it is the most inexpensive. Control experiments with no palladium catalyst or ligand were performed, but no product was formed, as expected.

Using the optimized reaction conditions we tested a broad range of aryl chlorides with different substituents, electron-donating (EDG) as well as electron-withdrawing groups (EWG), and with different disubstitution patterns (see Table 6). Both meta- and para-disubstituted aryl chlorides reacted efficiently giving full conversion after 1 h for most of the substrates. Electron-donating groups such as ethers, alcohols, and amines (entries 3, 6, and 7) worked well and the same was true for a variety of electron-withdrawing groups like nitriles, nitro groups, ketones, and esters (entries 10, 12, 13, and 15). Chlorobenzene (**25**) itself also reacted smoothly giving full conversion after 1 h (entry 1). Coupling of 4-chlorophenol (**29**), 4-chloroaniline (**30**), and 4-chlorobenzoic acid (**37**) did not proceed to completion after 1 h (entries 6, 7, and 14), but full conversion was achieved overnight (16 h). Apparently, the unprotected functional groups slowed down the reaction. Protection of the aniline nitrogen as in (*N*-Boc)-4-chloroaniline (**31**) gave full conversion after 1 h (entry 8). In the case of a free aliphatic amine (entry 9), the unprotected nitrogen completely quenched the reaction. To synthesize a halogen-substituted product, we tested the chemistry with 1-bromo-4-chlorobenzene (**39**) (entry 16) and the 4-chloro product **54** was formed exclusively. This is reasonable because bromides react faster than chlorides. Finally, we examined a couple of similar ortho-substituted aryl chlorides, but none of them gave any significant product formation after 16 h (entries 4 and 11). For the case of 2-chloroanisole (**27**) we also tested 2-bromoanisole (**28**) to examine if the more reactive bromide would react, but that was not the case (entry 5). It is not surprising that ortho-substituted aryl halides did not react at all, since there is much more steric hindrance around the halogen. Use of less sterically demanding ligands did not solve this problem.

In all cases, the crude product was isolated as the enol tautomer upon acidic workup. However, during flash chromatography we discovered some degree of shifting in equilibrium toward the keto tautomer, which gives more complex NMR spectra. To shift back the equilibrium we found that suspension in EtOAc and treatment with 10% KHSO<sub>4</sub> was suitable, which eventually dissolved the compound completely as the solubilities of the keto and enol tautomers are quite different. An example of this equilibrium shifting is shown in Figure 6 with the 3-aryl tetramic acid **3**. To the left is shown the enol tautomer and the appurtenant <sup>1</sup>H NMR spectrum in DMSO-*d*<sub>6</sub> and to the right the two possible keto tautomers. The <sup>1</sup>H NMR spectrum of **3**-keto is more complex because of broad peaks which might be a result of the coexistence of both a cis and trans tautomer. For both the enol and the keto tautomers, LC-MS (5→95% MeCN in H<sub>2</sub>O added 0.05% TFA) showed one peak with the same retention time. The acidic conditions apparently shift the equilibrium to one of the tautomers independent of the initial



**TABLE 6.** Substrate Scope with Different Substituted Aryl Chlorides

| entry | Ar-Cl | time (h) | product               | yield (%) <sup>d</sup>   |
|-------|-------|----------|-----------------------|--------------------------|
| 1     |       | 1        | <b>40</b>             | 75                       |
| 2     |       | 1        | <b>3</b>              | 79                       |
| 3     |       | 1        | <b>41</b>             | 78                       |
| 4     |       | 16       | <b>42</b>             | — <sup>b</sup>           |
| 5     |       | 1        | <b>43</b>             | — <sup>b</sup>           |
| 6     |       | 16       | <b>44</b>             | 72                       |
| 7     |       | 16       | <b>45</b>             | conv.: >95% <sup>c</sup> |
| 8     |       | 1        | <b>46</b>             | 75                       |
| 9     |       | 1        | <b>47</b>             | — <sup>b</sup>           |
| 10    |       | 1        | <b>48</b>             | 74                       |
| 11    |       | 16       | <b>49</b>             | — <sup>b</sup>           |
| 12    |       | 16       | <b>50</b>             | 60                       |
| 13    |       | 1        | <b>51</b>             | 74                       |
| 14    |       | 16       | <b>52</b>             | conv.: >95% <sup>c</sup> |
| 15    |       | 1        | <b>53</b>             | 75                       |
| 16    |       | 1        | <b>54<sup>d</sup></b> | 77                       |

<sup>a</sup> Purified by flash chromatography. <sup>b</sup> No significant product formation determined by <sup>1</sup>H NMR or LC-MS. <sup>c</sup> Not purified by flash chromatography, but crude <sup>1</sup>H NMR is provided in the Supporting Information. <sup>d</sup> Only the 4-chlorobenzene product was observed determined by the isotope pattern of the molecular ion (LC-MS) of the product (see the Supporting Information).

equilibrium position. In the <sup>1</sup>H NMR spectra (see Figure 6) the chemical shift of H<sup>5</sup> is 4.71 ppm (dd) for the enol tautomer but 4.28 ppm (m) for the keto tautomers (H<sup>5'</sup>). The coupling pattern of the H<sup>6</sup> diastereotopic protons also changes; for the enol-tautomer **3** the two protons are two well-resolved doublets (H<sup>6</sup><sub>a</sub> + H<sup>6</sup><sub>b</sub>), whereas the keto tautomers (**3**-keto) show a multiplet in the region slightly upfield (H<sup>6'</sup><sub>a</sub> + H<sup>6'</sup><sub>b</sub>). On the basis of <sup>1</sup>H NMR it was not possible to determine the ratio between the cis/trans tautomers.

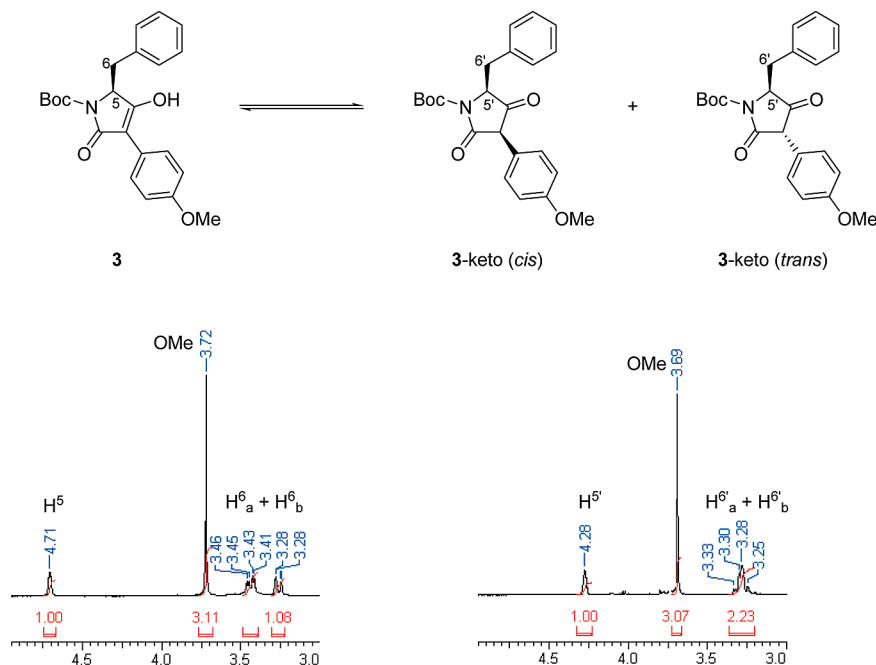
Besides the aryl chlorides we also examined the scope of the reaction with a series of chloro-substituted heterocycles (see Figure 7). Three different pyridines **55–57**, 2-chloropyrimidine (**58**), 5-chloro-1-methyl-1*H*-imidazole (**59**), two chloro thiophenes (**60** and **61**), and finally a bromo-substituted heterocycle, 3-bromothiophene (**62**), were tested. Unfortunately, none of them gave any significant formation of product after 16 h. It is plausible that the heteroatoms simply coordinate to palladium resulting in an unreactive complex.

The reaction between Boc-*py*Phe-OH (**1**) and 4-chloroanisole (**27**) was tested with microwave heating and it was found that full conversion (>95%) was achieved within only 5 min at 110 °C.

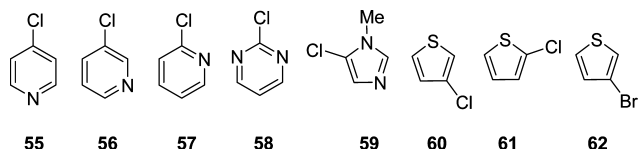
Finally, we wanted to expand the scope with other tetramic acids than Boc-*py*Phe-OH (**1**). A series of functionalized tetramic acids were chosen: Boc-*py*Tyr(<sup>t</sup>Bu)-OH (**63**), Boc-*py*Lys(Cbz)-OH (**64**), Boc-*py*Arg(Pbf)-OH (**65**), Boc-*py*Thr(O<sup>t</sup>Bu)-OH (**66**), Boc-*py*Asp(O<sup>t</sup>Bu)-OH (**67**), and the glycine-derived tetramic acid Boc-*py*Gly-OH (**68**). These were all subjected to the optimized reaction conditions with 4-chloroanisole (**21**) as the coupling partner (see Table 7). The chosen tetramic acids represent a broad variety of functional side chains and different protecting groups. Most of them gave similar yields compared to the previous results, but Boc-*py*Asp(<sup>t</sup>Bu)-OH (**67**) and especially Boc-*py*Gly-OH (**68**) gave much lower yield of the corresponding 3-aryl tetramic acids **73** and **74**, respectively (entries 5 and 6).

So far, all reactions were conducted in THF. We then revisited the initial reaction and tested some other solvents (see Table 8). Dioxane, MeCN, and DMF gave very low conversions (<5%) no matter which base was used (Cs<sub>2</sub>CO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, and K<sub>2</sub>CO<sub>3</sub>, respectively) (entries 1–3). However, running the reaction in toluene gave a significantly different result (entries 4–7). First of all, conversions were all much higher than the equivalent experiments in THF (cf. Table 1, entries 2–5), even with Na<sub>2</sub>CO<sub>3</sub> a conversion of 29% was achieved (see Table 8, entry 6). Full conversion was achieved with K<sub>3</sub>PO<sub>4</sub>, which gave only 36% conversion in THF with the very same ligand (**9**). Steric properties of the ligand are therefore not the only factor dependent on the efficiency of the catalytic system.

Surprisingly, the crude product isolated from the toluene/K<sub>3</sub>PO<sub>4</sub> reaction was exclusively the keto tautomer (**3**-keto cis/trans, see Figure 6), whereas THF gave the enol tautomer **3** when the same acidic workup procedure was used. To investigate this point further, we tried to shift the equilibrium of the enol tautomer **3** by dissolving it in toluene, but nothing happened based on TLC analysis. When adding aqueous 10% KHSO<sub>4</sub> the enol tautomer was slowly shifted toward the keto tautomers (**3**-keto cis/trans), which have a significantly different *R<sub>f</sub>* value. Isolation as the keto tautomers is unfortunately not always straightforward, and we discovered that it is easily shifted back to the enol tautomer. Formation of the less stable keto tautomers is not easy and it is rather unpredictable, probably because the



**FIGURE 6.** Observed tautomeric equilibrium and appurtenant <sup>1</sup>H NMR spectra (upfield region); the spectrum to the left belongs to the enol tautomer of 3 and the spectrum to the right to the keto tautomer (3-keto cis/trans).



**FIGURE 7.** Heteroaryl halides which did not undergo the title  $\alpha$ -arylation.

equilibrium shifting is dependent on concentration, temperature, pH, and solvent. This is comparable with the literature regarding the keto–enol equilibrium of tetramic acids,<sup>1,3,4</sup> as previously described.

On the basis of the literature,<sup>13a</sup> we propose the following mechanism for the coupling reaction (see Figure 8). The catalytic cycle is assumed to be initiated by reduction of Pd(II) to the active Pd(0), which might happen by a homocoupling of the tetramic acids. Oxidation of phosphine ligands is another well-known pathway for generation of Pd(0). However, Barder and Buchwald reported recently that dialkylbiaryl phosphines are highly resistant toward oxidation by molecular oxygen.<sup>17</sup> Following reduction of Pd(II) to Pd(0), oxidative addition of the aryl halide 25 takes place, then transmetalation by the potassium enolate of the tetramic acid 1. Upon reductive elimination the product is released and Pd(0) re-enters the catalytic cycle. The desired product 40 can be isolated by acidic workup.

In conclusion, we have developed a new, mild, and racemization-free palladium-catalyzed  $\alpha$ -arylation of tetramic acids giving rise to 3-aryl amino acid-derived tetramic acids. Through optimization it was found that 2 mol % of Pd(OAc)<sub>2</sub> and 4 mol % of 2-di-*tert*-butylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl (12) gave full conversion in THF at 80 °C after 1 h for most substrates. The two weak inorganic potassium bases, K<sub>2</sub>CO<sub>3</sub> and K<sub>3</sub>PO<sub>4</sub>, worked equally well. The product could be isolated as either the enol or the keto tautomer depending on reaction

solvent and the workup conditions, but shifted back to the enol tautomer upon EtOAc/10% KHSO<sub>4</sub> treatment. A variety of different substrates was tested and a range of functionalities are tolerated, e.g., Boc- or Cbz-protected amines, Pbf-protected guanine groups, ethers, esters, ketones, alcohols, nitriles, and nitro groups. Heterocycles and unprotected amines are not compatible with this chemistry. Aryl chlorides, bromides, and triflates all coupled nicely, whereas aryl iodides and tosylates did not work. With respect to the substitution pattern of the aryl chloride, electron-withdrawing as well as electron-donating groups showed similar reactivity and meta- and para-substituted aryl chlorides reacted identically. Due to steric hindrance ortho-substituted aryl chlorides did not react. The title reaction can be facilitated by microwave heating with reaction time down to 5 min at 110 °C.

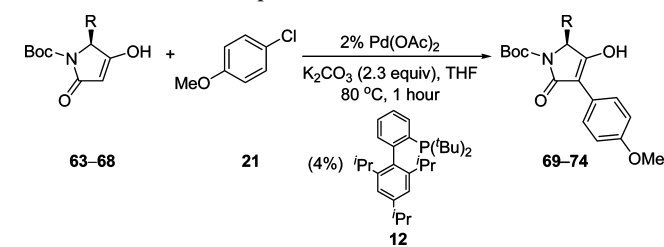
Synthesis of the previously mentioned amino benzyl ketones is currently under development in our laboratory. These building blocks will ultimately be used for the preparation of C-terminal modified peptidyl enzyme inhibitors.

## Experimental Section

**General Arylation Procedure.** A vial was charged with dry THF (3.0 mL), tetramic acid (1.00 mmol, 1.00 equiv), 2-di-*tert*-butylphosphino-2',4',6'-triisopropylbiphenyl (12) (17 mg, 0.04 mmol, 0.04 equiv), K<sub>2</sub>CO<sub>3</sub> (318 mg, 2.30 mmol, 2.30 equiv), and an aryl chloride (1.00 mmol, 1.00 equiv). N<sub>2</sub> was bubbled through the reaction mixture and Pd(OAc)<sub>2</sub> (4 mg, 0.02 mmol, 0.02 equiv) was added, then the vial was filled with N<sub>2</sub>, sealed with a screw cap, and placed in an aluminum heating block. The mixture was stirred vigorously at 80 °C for 1 h (or 16 h, cf. Tables 6 and 7). After being cooled to ambient temperature, the crude mixture was transferred to a separatory funnel with 10% aqueous KHSO<sub>4</sub> (10 mL) and extracted with EtOAc (30 mL + 20 mL). The combined organic phases were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated in vacuo. The yellow crude product was purified by flash chromatography (5→10% MeOH in EtOAc, in some cases up to 20% MeOH) affording the pure product typically as a keto/enol tautomer mixture. The product was subsequently suspended

(17) Barder, T. E.; Buchwald, S. L. *J. Am. Chem. Soc.* **2007**, *129*, 5096–5101.

TABLE 7. Substrate Scope with Different Tetramic Acids



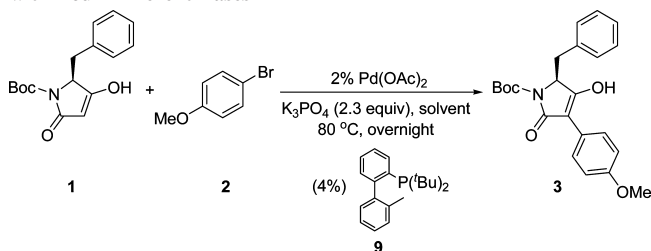
| entry | tetramic acid | time (h) | product   | yield (%) <sup>a</sup> |
|-------|---------------|----------|-----------|------------------------|
| 1     |               | 1        | <b>69</b> | 69                     |
| 2     |               | 1        | <b>70</b> | 69                     |
| 3     |               | 16       | <b>71</b> | 67                     |
| 4     |               | 1        | <b>72</b> | 62                     |
| 5     |               | 16       | <b>73</b> | 45                     |
| 6     |               | 16       | <b>74</b> | 28 <sup>b</sup>        |

<sup>a</sup> Purified by flash chromatography. <sup>b</sup> Flash chromatography did not successfully purify the product.

in EtOAc (50 mL). Ten percent aqueous  $\text{KHSO}_4$  (50 mL) was added and the biphasic system was stirred vigorously at room temperature until complete dissolution of the compound. The mixture was transferred to a separatory funnel and the organic layer was separated. The aqueous layer was extracted with EtOAc (20 mL) and the combined organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, evaporated in vacuo, and dried overnight in high vacuum, which afforded the pure product mostly as the enol tautomer.

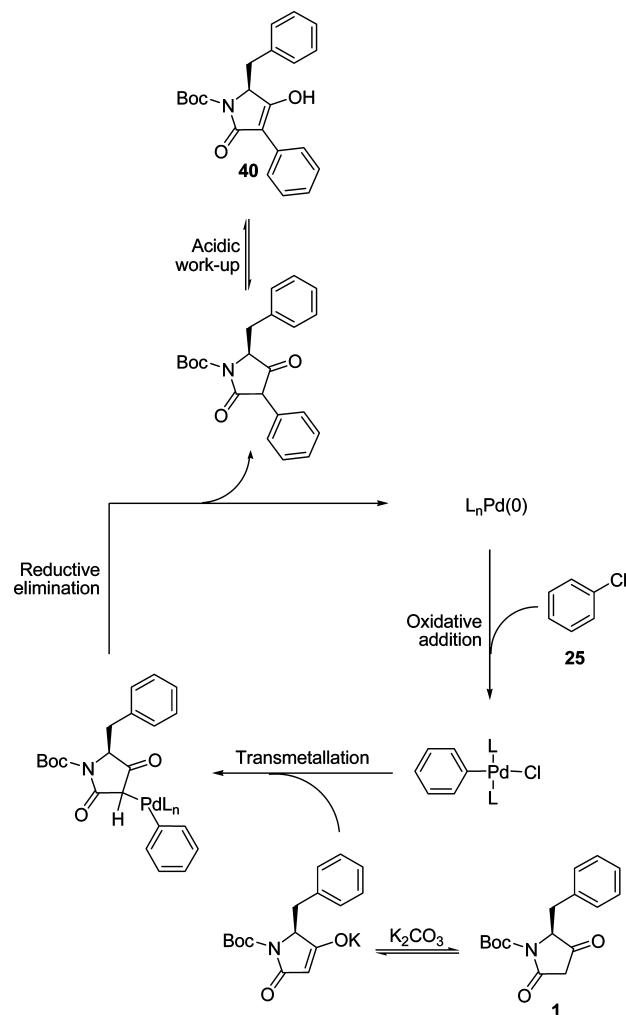
**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydropyrrol-2-one (3).** Following the general method for the arylation afforded 79% (313 mg) of the desired product as a pale brown solid.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  12.11 (br s,

TABLE 8. Initial Reaction Conducted in Toluene and Screened with Four Different Bases



| entry | solvent | base                     | conv (%) <sup>a</sup> |
|-------|---------|--------------------------|-----------------------|
| 1     | dioxane | all four <sup>b</sup>    | <5                    |
| 2     | MeCN    | all four <sup>b</sup>    | <5                    |
| 3     | DMF     | all four <sup>b</sup>    | <5                    |
| 4     | toluene | $\text{Cs}_2\text{CO}_3$ | 86                    |
| 5     | toluene | $\text{K}_3\text{PO}_4$  | >95                   |
| 6     | toluene | $\text{Na}_2\text{CO}_3$ | 29                    |
| 7     | toluene | $\text{K}_2\text{CO}_3$  | 88                    |

<sup>a</sup> Determined by  $^1\text{H}$  NMR. <sup>b</sup> Tested with the four bases  $\text{Cs}_2\text{CO}_3$ ,  $\text{K}_3\text{PO}_3$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{K}_2\text{CO}_3$ , respectively.

FIGURE 8. Proposed reaction mechanism for the  $\alpha$ -arylation of tetramic acids.

1H), 7.48 (d,  $J = 8.6$  Hz, 2H), 7.21–7.13 (m, 3H), 6.98 (d,  $J = 6.8$  Hz, 2H), 6.86 (d,  $J = 8.8$  Hz, 2H), 4.71 (dd,  $J = 4.3, 2.3$  Hz, 1H), 3.72 (s, 3H), 3.44 (dd,  $J = 14.0, 4.9$  Hz, 1H), 3.26 (dd,  $J = 13.8, 1.9$  Hz, 1H), 1.53 (s, 9H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  168.9, 167.6, 157.7, 149.0, 134.2, 129.5, 128.7, 127.9, 126.8, 122.9, 113.2,

105.1, 81.2, 57.9, 55.0, 34.6, 27.9. HRMS ( $m/z$ ) calcd for  $C_{46}H_{50}N_2O_{10}Na$   $[2M + Na]^+$  813.3358, found 813.3367. Anal. Calcd for  $C_{23}H_{25}NO_5$ : C, 69.86; H, 6.37; N, 3.54. Found: C, 69.84; H, 6.52; N, 3.49. Mp 142–145 °C. IR (neat)  $\nu$  2975, 2930, 1750 (strong), 1363, 1284, 1251, 1147, 1095, 833, 699  $cm^{-1}$ . Chiral HPLC: 4.57 min (minor) and 5.78 min (major) gave an enantiomeric excess of 97%.

**Microwave-Assisted Synthesis of (5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydropyrrol-2-one (3).** A microwave vial was charged with Boc-pyPhe-OH (**1**) (289 mg, 1.00 mmol, 1.00 equiv), 2-di-*tert*-butylphosphino-2',4',6'-triisopropylbiphenyl (**12**) (17 mg, 0.04 mmol, 0.04 equiv),  $Pd(OAc)_2$  (4 mg, 0.02 mmol, 0.02 equiv), and  $K_2CO_3$  (318 mg, 2.30 mmol, 2.30 equiv). Dry THF (3.0 mL) was added and used to carefully rinse the inside of the vial (for safety reasons no solid may be stuck on the glass!). 4-Chloroanisole (**21**) (143 mg, 0.08 mL, 1.00 equiv) was added and  $N_2$  was bubbled into the vial to secure an inert reaction atmosphere. The vial was capped, sealed, and heated to 110 °C in a microwave synthesizer for 5 min. After being cooled to ambient temperature, the crude product was neutralized with 10%  $KHSO_4$  (10 mL) and EtOAc (30 + 20 mL) was added. The combined organic layers were dried over anhydrous  $Na_2SO_4$  and evaporated in vacuo. The crude product was analyzed by  $^1H$  NMR in DMSO- $d_6$ , which showed full conversion (>95%), and the spectrum was identical with that of the product obtained by conventional heating (80 °C, 1 h).

**(5S)-5-Benzyl-1-(tert-butyloxycarbonyl)-4-hydroxy-3-phenyl-1,5-dihydropyrrol-2-one (40).** Following the general method for the arylation afforded 75% (273 mg) of the desired product as a pale brown solid.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  12.30 (br s, 1H), 7.52 (dd,  $J$  = 8.3, 1.3 Hz, 2H), 7.28 (t,  $J$  = 7.6 Hz, 2H), 7.22–7.15 (m, 4H), 7.01–6.98 (m, 2H), 4.73 (dd,  $J$  = 4.8, 2.5 Hz, 1H), 3.45 (dd,  $J$  = 13.9, 4.8 Hz, 1H), 3.27 (dd,  $J$  = 14.0, 2.4 Hz, 1H), 1.54 (s, 9H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  170.2, 167.4, 149.0, 134.2, 130.5, 129.5, 127.9, 127.7, 127.5, 126.8, 126.3, 105.4, 81.2, 57.9, 34.6,

27.9. HRMS ( $m/z$ ) calcd for  $C_{44}H_{46}N_2O_8Na$   $[2M + Na]^+$  753.3146, found 753.3153. Anal. Calcd for  $C_{22}H_{23}NO_4$ : C, 72.31; H, 6.34; N, 3.83. Found: C, 71.97; H, 6.60; N, 3.96. Mp 86–88 °C. IR (neat)  $\nu$  3082, 3061, 2977, 2928, 1753, 1702, 1661, 1645 (strong), 1397, 1359, 1298, 1149, 694  $cm^{-1}$ .

**(5S)-5-(4-*tert*-Butoxybenzyl)-1-(tert-butyloxycarbonyl)-4-hydroxy-3-(4-methoxyphenyl)-1,5-dihydropyrrol-2-one (69).** Following the general method for the arylation afforded 69% (324 mg) of the desired product as a pale brown solid.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  12.02 (s, 1H), 7.42 (d,  $J$  = 9.1 Hz, 2H), 6.88 (d,  $J$  = 8.6 Hz, 2H), 6.83 (d,  $J$  = 9.1 Hz, 2H), 6.77 (d,  $J$  = 8.6 Hz), 4.66 (dd,  $J$  = 4.6, 2.8 Hz, 1H), 3.71 (s, 3H), 3.39 (dd,  $J$  = 13.9, 4.6 Hz, 1H), 3.19 (dd,  $J$  = 13.9, 2.5 Hz, 1H), 1.53 (s, 9H), 1.16 (s, 9H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  168.8, 167.6, 157.7, 153.7, 149.0, 130.0, 128.9, 128.7, 123.2, 122.8, 113.1, 105.4, 81.1, 77.7, 57.9, 55.0, 34.1, 28.4, 27.9. HRMS ( $m/z$ ) calcd for  $C_{54}H_{66}N_2O_{12}Na$   $[2M + Na]^+$  957.4508, found 957.4516. Mp 147–153 °C. IR (neat)  $\nu$  2975, 2932, 1748, 1643, 1607, 1514, 1392, 1363, 1290, 1247, 1150 (strong), 1095, 894, 830  $cm^{-1}$ .

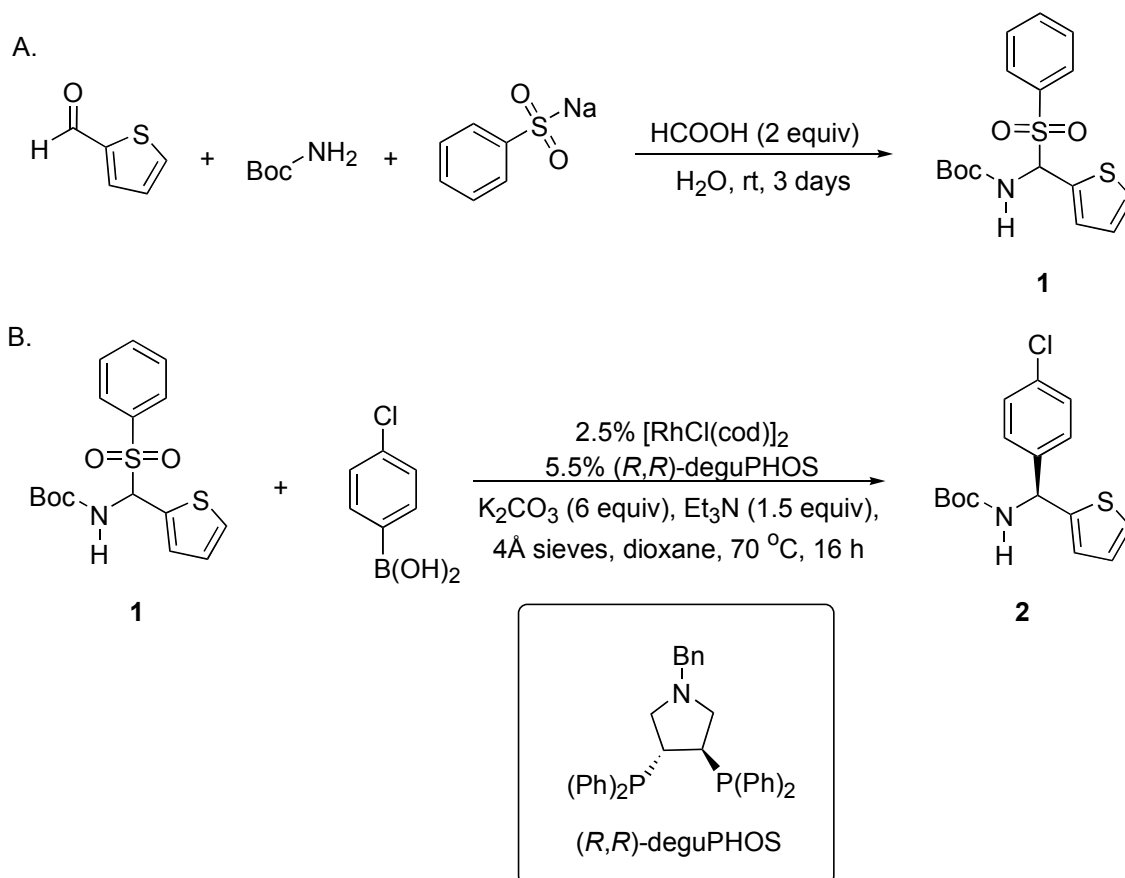
**Acknowledgment.** Morten Storgaard thanks Novo Nordisk A/S, Corporate Research Affairs, and the Danish Ministry of Science, Technology and Innovation for financial support.

**Supporting Information Available:** Experimental procedures and characterization of all new compounds except those mentioned in the Experimental Section (compounds **3**, **40**, and **69**) and copies of  $^1H$  and  $^{13}C$  NMR spectra (including  $^1H$  NMR spectra of compounds **45** and **52**), analytical HPLC chromatograms of compounds not provided with elemental analysis, chiral HPLC chromatogram of compound **3**, and MS (TOF ES+) of compound **54**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO900799Y



# RHODIUM-CATALYZED ENANTIOSELECTIVE ADDITION OF ARYLBORONIC ACIDS TO *IN SITU* GENERATED *N*-BOC ARYLIMINES. PREPARATION OF (*S*)-*TERT*-BUTYL (4-CHLOROPHENYL)(THIOPHEN-2-YL)METHYLCARBAMATE



Submitted by Morten Storgaard and Jonathan A. Ellman.<sup>1</sup>  
 Checked by Jason A. Bexrud and Mark Lautens.

## 1. Procedure

*A. tert-Butyl phenylsulfonyl(thiophen-2-yl)methylcarbamate (1).* In a 250-mL, round-bottomed flask benzenesulfinic acid sodium salt (13.13 g, 80.0 mmol, 2.0 equiv) (Note 1) is dissolved in H<sub>2</sub>O (105 mL) (Note 2). *tert*-Butyl carbamate (4.69 g, 40.0 mmol, 1.0 equiv) (Note 3) is added, but does not dissolve. 2-Thiophene-carboxaldehyde (5.50 mL, 6.73 g, 60.0 mmol, 1.5 equiv) (Note 4) is added forming a yellow emulsion. Formic acid (3.10 mL, 3.68 g, 80.0 mmol, 2.0 equiv) (Note 5) is added. The flask is loosely fitted with a rubber septum and the white, opaque, biphasic mixture is stirred

vigorously at room temperature (23 °C). After a couple of hours the water phase becomes clear. The product **1** is formed as yellow chunks, which become more dispersed in the water phase as the reaction proceeds. After 3 days (Note 6) of stirring the suspension is vacuum filtered (Note 7). The yellow chunks are crushed with a spatula, and the product is triturated with H<sub>2</sub>O (2 × 10 mL) and Et<sub>2</sub>O (2 × 10 mL) (Note 8). After each trituration the solvent is removed by vacuum filtration. Finally, it is dried for an hour under high vacuum (Note 9) to give the imine precursor **1** as a white solid (8.21 g, 58%) (Notes 10 and 11).

*B. (S)-tert-Butyl (4-chlorophenyl)(thiophen-2-yl)methylcarbamate (2).* An oven-dried (Note 12), 250-mL, three-necked round-bottomed flask with a magnetic stir bar is equipped with a vacuum adaptor in the middle neck and glass stoppers in the two other necks (one of which is loosely fitted to allow an outflow of nitrogen gas). The adaptor is connected to a nitrogen gas line (Note 13) and the flask is purged with nitrogen as it is allowed to cool to ambient temperature (23 °C). The flask is then charged with [RhCl(cod)]<sub>2</sub> (247 mg, 0.50 mmol, 0.025 equiv) (Note 14) and (*R,R*)-deguPHOS (583 mg, 1.1 mmol, 0.055 equiv) (Note 15) by removing one of the glass stoppers. A septum is used to seal the flask and the other glass stopper is exchanged with an adaptor equipped with a thermometer. The flask is then purged with nitrogen for 5 min and a positive nitrogen flow is thereafter maintained to ensure an oxygen-free atmosphere inside the flask (Note 16). Dry dioxane (80 mL) (Note 17) is added through the septum via a syringe and the flask is submerged into an oil bath (70 °C), and the mixture is stirred for 1 h (internal temperature: 65 °C, reached after 20 min). Initially, the precatalyst is not fully soluble in dioxane, but as the preincubation proceeds it completely dissolves. The solution of the active catalyst is clear and dark orange.

Meanwhile (Note 18), a 500-mL, oven-dried, three-necked round-bottomed flask (Note 19) with a magnetic stir bar is equipped with a vacuum adaptor in the middle neck and glass stoppers in the two other necks (one of which is loosely fitted to allow outflow of nitrogen gas). The adaptor is connected to a nitrogen gas line and the flask is purged with nitrogen as it is allowed to cool to ambient temperature (23 °C). The flask is then charged with *tert*-butyl phenylsulfonyl(thiophen-2-yl)methylcarbamate (**1**) (7.07 g, 20.0 mmol, 1.0 equiv), 4-chlorophenylboronic acid (6.26 g, 40.00 mmol, 2.0 equiv) (Notes 20 and 21), K<sub>2</sub>CO<sub>3</sub> (16.58 g, 120.0 mmol, 6.0 equiv) (Note 22) and 4Å powdered molecular sieves (32 g) (Note 23) by removing one of the

glass stoppers. A septum is used to seal the flask, and the other glass stopper is exchanged with an adaptor equipped with a thermometer. The flask is then purged with nitrogen for 5 min, and a positive nitrogen inflow is maintained to ensure an oxygen-free atmosphere inside the flask. Dry dioxane (240 mL) is added through the septum via a syringe immediately before the preincubation is complete (described above). Additionally, dry triethylamine (4.20 mL, 3.04 g, 30.00 mmol, 1.5 equiv) (Note 24) is added via a syringe. The white suspension is stirred vigorously at room temperature (23 °C) while adding the preincubated solution of catalyst and ligand via cannula transfer (Note 25) resulting in a yellow suspension. The reaction flask is submerged into an oil bath (70 °C), and the yellow suspension is stirred vigorously for 16 h (internal temperature: 70 °C) (Note 26). The yellow suspension is allowed to cool to ambient temperature (23 °C) over the course of one hour and vacuum filtered through a plug of Celite<sup>TM</sup> (Note 27), which is rinsed with EtOAc (300 mL) (Note 28). The combined yellow filtrates are evaporated *in vacuo* (Note 29) to give a yellow solid (Note 30). The crude product is purified by flash chromatography (6.5 × 20 cm, 270 g silica gel) (Note 31) using a gradient of 5 to 15% EtOAc in hexanes and fractions of 50 mL. The column is eluted with 500 mL of 1:19 EtOAc:hexanes (Note 32), 500 mL of 1:12 EtOAc:hexanes, 1500 mL of 1:9 EtOAc:hexanes, 500 mL of 1:7 EtOAc:hexanes and finally with 500 mL of 1:5 EtOAc:hexanes. Fractions 32–65 (Note 33) are combined, evaporated *in vacuo* and dried overnight under high vacuum affording the title compound **2** as a white solid (4.92 g, 76%) (Notes 34 and 35) with 93% ee (Notes 36 and 37).

## 2. Notes

1. Benzenesulfinic acid sodium salt (98%) was purchased from Sigma-Aldrich and used without further purification.
2. Deionized water (H<sub>2</sub>O) was used in all cases where the procedures call for water.
3. *tert*-Butyl carbamate (98%) was purchased from Sigma-Aldrich and was used without further purification.
4. 2-Thiophene-carboxaldehyde (98%) was purchased from Sigma-Aldrich and was used without further purification.
5. Formic acid (HCOOH) (reagent grade, >95%) was purchased from Sigma-Aldrich and was used without further purification.

6. This reaction was originally published by Wenzel and Jacobsen<sup>2</sup> giving only a 44% yield of product **1** when MeOH:H<sub>2</sub>O (1:2) was used as the solvent and with a 3 day reaction time. We attempted to increase the yield by heating the reaction mixture to 50 °C, but this resulted in product decomposition. Increasing the reaction concentration resulted in only a slight increase in the yield of **1**. Reducing the amount of MeOH resulted in the most significant increase in yield. Ultimately, running the reaction in pure H<sub>2</sub>O gave the reported yield. Lower yields were achieved with a reaction time of only 1 day (44%), while a further increase in the yield can be achieved after 5 days (74%).

7. Wilmad Labglass sintered glass funnel, 60 mL, size M, was used.

8. Diethyl ether (Et<sub>2</sub>O), anhydrous HPLC grade, stabilized, was purchased from Fisher Scientific Chemicals and was used without further purification.

9. High vacuum refers to 0.025 mmHg at 23 °C.

10. *tert*-Butyl phenylsulfonyl(thiophen-2-yl)methylcarbamate (**1**) exhibits the following properties: mp 160–162 °C (decomp.). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.26 (s, 9 H), 5.62 (d, *J* = 10.0 Hz, 1 H), 6.18 (d, *J* = 10.8 Hz, 1 H), 7.05–7.09 (m, 1 H), 7.26–7.28 (m, 1 H), 7.41–7.43 (m, 1 H), 7.52–7.57 (m, 2 H), 7.63–7.65 (m, 1 H), 7.90–7.94 (m, 2 H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 28.2, 70.4, 81.7, 127.6, 128.0, 129.3, 129.6, 129.8, 131.7, 134.3, 136.7, 153.4. IR (neat) 3347, 2955, 1699, 1510, 1306, 1150 cm<sup>-1</sup>. Anal. calcd for C<sub>16</sub>H<sub>19</sub>NO<sub>4</sub>S<sub>2</sub>: C, 54.37; H, 5.42; N, 3.96; found: C, 54.39; H, 5.40; N, 3.87.

11. A second run by the checkers provided 10.57 g (75%) of **1** with a melting point range of 159–161 °C. The submitters reported a yield of 8.46 g (60%) with a melting point range of 162–164 °C.

12. Oven-dried refers to drying of flasks, glass stoppers, adaptors and magnetic stir bars in an oven (150 °C) overnight before use. The glassware was assembled while still hot and cooled to ambient temperature (23 °C) under high vacuum. The submitters cooled the glassware under high vacuum.

13. The nitrogen gas line was a standard dual manifold with multiple ports with stopcocks that allow vacuum or nitrogen to be selected without the need for placing the flask on a separate line. One manifold was connected to a source of nitrogen dried through a tube of Drierite<sup>®</sup> (>98% CaSO<sub>4</sub>, >2% CoCl<sub>2</sub>), while the other was connected to a high-vacuum Fisher Scientific Maxima<sup>®</sup> C Plus Model M8C pump (0.025 mm Hg). The nitrogen

gas line was vented through an oil bubbler that was connected to the manifold through a valve (making it possible to disconnect the bubbler during cannula transfer), while solvent vapors were prevented from contaminating the pump through a dry ice/isopropanol cold trap.

14.  $[\text{RhCl}(\text{cod})]_2$  (chloro(1,5-cyclooctadiene)rhodium(I) dimer), 98%, which is air stable, was purchased from Strem and was used without further purification.

15. (*R,R*)-deguPHOS ((3*R*,4*R*)-1-benzyl-3,4-bis-diphenylphosphanylpyrrolidine), 98%, was purchased from Strem and was used without further purification.

16. The active catalyst is very sensitive to air. It is important to introduce a nitrogen atmosphere to the flask and maintain a positive pressure of nitrogen throughout the preincubation and the reaction to prevent catalyst decomposition.

17. 1,4-Dioxane, HPLC grade, was purchased from Fisher Scientific Chemicals and passed through a column of dry, activated, basic alumina under a nitrogen atmosphere. The solvent is transferred to the flask via a syringe without exposure to air.

18. This part of the procedure can be performed while stirring the precatalyst and ligand, but the dioxane and  $\text{Et}_3\text{N}$  should not be added until just immediately before the active catalyst is ready (1 h at 70 °C). This is to avoid premature hydrolysis of the *in situ* generated imine, which results in a decreased yield of the title compound **2**.

19. A 500-mL flask was used instead of a 1000-mL to reduce the risk of catalyst decomposition – minimizing unoccupied volume reduces the risk of oxygen contamination.

20. 4-Chlorophenylboronic acid (95%) was purchased from Sigma-Aldrich and recrystallized from  $\text{H}_2\text{O}$  before use as described in Note 21.

21. Commercially available arylboronic acids contain boroximes (anhydride trimers) that do not add efficiently to the *in situ* generated imine. Therefore, to maximize formation of the title compound **2**, we found it very important to recrystallize and dry the arylboronic acid before use. This was carried out as follows: in a 1000-mL conical flask was added 4-chlorophenylboronic acid (10 g) (Note 20) and  $\text{H}_2\text{O}$  (400 mL) and the flask was covered with a watch glass. The suspension was heated to boiling over the course of 25 minutes on a heating plate (115 °C) under vigorous stirring with a magnetic stir bar. The boiling point was maintained for 5 minutes to fully dissolve the boronic acid. The hot solution was filtered through filter

paper using gravity filtration to remove insoluble particles. The colorless solution was cooled to ambient temperature (23 °C) overnight and then was cooled in an ice bath for 1 hour (internal temperature: 5 °C). During the cooling process the arylboronic acid precipitated and was isolated by vacuum filtration and dried by continuing the vacuum filtration for an additional 15 minutes. To remove further amounts of water the boronic acid was dried in high vacuum at room temperature (23 °C) until  $^1\text{H}$  NMR analysis in  $\text{DMSO-}d_6$  showed a composition of no more than approx. 5% boroxime and 30%  $\text{H}_2\text{O}$ , at which point the mass of product was 8.0–8.6 g of white microplates. The drying procedure is important because reaction of pure boroxime will cause a reduction in the yield of the title compound **2** down to 52%. Depending on the initial amount of water in the recrystallized batch and the vacuum pump capacity, the time of drying may vary. Usually we were able to obtain the above-mentioned composition requirements within 5–15 minutes of drying in high vacuum. It is highly recommended to dry the arylboronic stepwise, e.g. 5 minutes at a time and then analyze the arylboronic by NMR. In  $\text{DMSO-}d_6$  (dried prior to use over 4Å molecular sieves, 3.2 mm pellets) 4-chlorophenylboronic acid exhibits the following chemical shifts (300 MHz):  $\delta$  8.16 (s, broad), 7.79 (d,  $J$  = 8.3, 2H), 7.39 (d,  $J$  = 8.3, 2H), while the corresponding boroxime exhibits these shifts:  $\delta$  7.86 (d,  $J$  = 8.1, 2H), 7.42 (d,  $J$  = 8.1, 2H). The composition can be determined using the integrals directly if the DMSO is water-free. Occasionally, we found it difficult to remove the excess of water without increasing the amount of boroxime to strictly more than 5%. In such cases the batch should be recrystallized again.

22. Potassium carbonate ( $\text{K}_2\text{CO}_3$ ), anhydrous, was purchased from EM Science (an affiliate of Merck KGaA) and was dried overnight before use under high vacuum at 100 °C in a thermostatically controlled oil bath.

23. Molecular sieves, 4Å, <5 microns, powdered, were purchased from Sigma-Aldrich and activated under high vacuum at 230–260 °C overnight. Heating was achieved by a Glas-Col<sup>®</sup> heating mantle, 2/3 filled with sand and connected to a Powerstat<sup>®</sup> variable autotransformer (in: 120 V, 50/60, ~1 PH, out: 0–140 V, 10 A, 1.4 KVA). The transformer was adjusted to approx. 250 °C as measured with a thermometer placed directly into the sand.

24. Triethylamine was purchased from Fisher Scientific Chemicals and was freshly distilled from  $\text{CaH}_2$  under a nitrogen atmosphere before use.

25. Cannulation technique (Figure 1) was used to conveniently transfer the active catalyst solution (A) to the mixture of starting materials, bases and molecular sieves (B) through a cannula (C) without exposure to air. Before inserting the cannula into the flasks, an extra oil bubbler (D) was attached to flask B via a needle through the septum. The cannula (C) was then inserted into flask A and after a minute the other end of C was inserted into flask B. To cannulate the catalyst solution (A), the nitrogen inlet to flask B (E) and the Schlenk line oil bubbler (F) were both closed making the extra oil bubbler (D) the only outlet from the system. After complete cannulation E and F were both opened again, and the extra bubbler (D) and cannula (C) were removed.

26. It is not convenient to monitor the progress of the reaction by TLC because the diagnostic compound (the imine formed *in situ* from **1**) is unstable and does not elute without decomposition on TLC. Therefore, we ran a number of reactions on small scale (0.250 mmol) at different reaction times. We found that the amount of the title product **2** reaches a 65–67% NMR yield after 10 hours. Neither product decomposition nor an increase in yield are observed with prolonged reaction times, e.g., 40 hours at 70 °C. For convenience we chose a 16 h reaction time.

27. Celite<sup>TM</sup> powder, 545 filter aid, not acid washed, was purchased from Fisher Scientific and was used without further purification. The filter plug was prepared by mixing Celite<sup>TM</sup> (20 g) with EtOAc (80 mL) and filtered through a Kimax<sup>®</sup> sintered glass funnel, 150 mL – 60F.

28. Ethyl acetate (EtOAc), HPLC grade, was purchased from Fisher Chemicals and was used without further purification.

29. Evaporation *in vacuo* was carried out on a Büchi Rotavapor R-114 at 45 mmHg with a Büchi Waterbath B-480 at 35 °C, unless otherwise stated.

30. The <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of the crude product was recorded to determine if the reaction proceeded as expected. Besides EtOAc, dioxane and the peaks corresponding to the title compound **2** (Note 34), the crude product also contains 2-thiophene-carboxyaldehyde (9.68 ppm), and other decomposition compounds: δ 7.86, 7.75, 4.57 and 1.28 ppm. If the reaction has been performed correctly there should be only trace amounts of the *in situ* generated imine (9.05 and 1.57 ppm) in the crude product, and the crude product should be a yellow solid rather than an oil.

31. Silica gel 60 (0.040–0.063 mm), 230–400 mesh ASTM, was purchased from Merck KGaA and used without further purification.

32. Hexanes, HPLC grade, was purchased from Fisher Scientific and used without further purification.
33. TLC of fractions is performed using Dynamic Adsorbents, Inc. glass plates coated with 250 mm F-254 silica gel. 15% EtOAc in hexanes is used as the eluent. Visualization is achieved with UV (Spectroline<sup>®</sup>, Model EF-140C, short wave ultraviolet 254 nm) and subsequently with PMA staining (10 g phosphomolybdic acid + 100 mL absolute EtOH) by immersion and heating with a heat gun. The title compound **2** is visible by UV and stains dark brown with PMA at an  $R_f = 0.41$ . Trace amounts of 2-thiophene-carboxaldehyde, which is generated by decomposition of *tert*-butyl phenylsulfonyl(thiophen-2-yl)methylcarbamate (**1**), elutes at  $R_f = 0.35$ . This aldehyde is only visible by UV and does not stain with PMA. Fractions containing both thiophene-carboxaldehyde and **2** are collected because this aldehyde is easy to remove under vacuum (boils at 75 – 77 °C at 11 mmHg). Fractions containing an impurity with  $R_f = 0.22$  (visible by UV and stains brown with PMA) were not collected.
34. The title compound (**2**) exhibits the following properties: mp 138–140 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.43 (s, 9 H), 5.20 (broad s, 1 H), 6.10 (broad s, 1 H), 6.77–6.80 (m, 1 H), 6.90–6.94 (m, 1 H), 7.22–7.34 (m, 5 H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 28.6, 54.2, 80.5, 125.5, 125.9, 127.1, 128.5, 129.0, 133.7, 140.6, 145.9, 154.9. IR (neat) 3347, 2979, 2921, 2361, 1686, 1515, 1233, 1169 cm<sup>-1</sup>.  $[\alpha]_D^{20} +11.0$  ( $c = 0.5$ , EtOH). MS (ESI+)  $m/z$  346 ( $M^+ + Na$ , 100%), 347 (17%), 348 (40%). Anal. calcd. for C<sub>16</sub>H<sub>18</sub>ClNO<sub>2</sub>S: C, 59.34; H, 5.60; N, 4.33; found: C, 59.50; H, 5.63; N, 4.23.
35. The checkers also performed the reaction at half-scale and isolated pure product in a 65% yield at 96% ee. The submitters report a full-scale reaction to provide product in 65% yield at 95-99% ee.
36. The absolute configuration was shown by anomalous dispersion to be (*S*) using X-ray crystallography. This configuration is consistent with prior additions of this type [see Reference 13].
37. Enantiomeric excess is determined by chiral HPLC using an Agilent 1100 series instrument and a Chiralpak<sup>®</sup> AS-H column (amylose tris[(*S*)- $\alpha$ -methylbenzyl-carbamate] coated on 5 mm silica gel), L = 250 mm, I.D. = 4.6 mm, from Danicel Technologies, LTD. 1% EtOH in hexanes is used as the eluent (isochratic) with a flow rate of 1.00 mL/min (max. 70 bar) for 25 minutes. For optimal performance the column is equilibrated with the solvent system for at least 45 minutes before running the sample. A sample



is prepared by dissolving approx. 1 mg compound in 1 mL of 1% EtOH in hexanes and filtering through a 4 mm nylon syringe filter (0.45 mm) purchased from National Scientific. 5.0 mL of this solution is used for injection. To determine the retention times for both enantiomers, a racemate of **2** (synthesized with dppBenz as ligand) can be analyzed: (*R*)-enantiomer (minor): 11.1 minutes and (*S*)-enantiomer (major): 13.5 minutes. Samples are analyzed at the following wavelengths: 222, 230, 250 and 254 nm each of which gave similar %ee.

### Waste Disposal Information

All hazardous materials should be handled and disposed of in accordance with “Prudent Practices in the Laboratory”; National Academy Press; Washington, DC, 1995.

### 3. Discussion

Synthesis of enantiomerically pure functionalized amines is of great importance because such compounds are widely used in drugs. The rhodium-catalyzed enantioselective addition of arylboronic acids to *in situ* generated *N*-Boc aromatic imines is a general and easy method for the preparation of *N*-Boc protected diaryl methanamines. The first reported example of addition of arylboronic acids to an imine was the addition to *N*-sulfonyl aldimines published in 2000 by Miyaura and co-workers.<sup>3</sup> A number of enantioselective variants were later developed using chiral ligands, such as *N*-Boc-L-valine amidomonophosphanes,<sup>4</sup> (1*R*,4*R*)-bicyclo[2.2.2]-octadienes,<sup>5</sup> (*S*)-ShiP,<sup>6</sup> monodentate phosphoramidites,<sup>7,8</sup> binaphtholic phosphites,<sup>8</sup> tetrahydropentalenes,<sup>9</sup> and (*R,R*)-deguPHOS.<sup>10</sup> Most of the methods are limited to aromatic imines, but more recently enantioselective catalytic additions to aliphatic imines have also been reported.<sup>11,12</sup>

However, these methods suffer from a number of drawbacks. For example, all of the methods utilize unstable imine substrates, and many of the methods necessitate the use of very harsh conditions to remove the *N*-substituent present in the addition products. Some of these problems were previously solved by the Ellman group using *N*-Boc aromatic imines generated *in situ* from easily prepared and stable  $\alpha$ -carbamoyl sulfones in an enantioselective addition with arylboronic acids (Table 1).<sup>13</sup> Commercially

available (*R,R*)-deguPHOS was used as the chiral ligand to obtain enantiomeric excesses up to 99%. However, Rh(acac)(coe)<sub>2</sub> was used as the precatalyst, and it is currently not commercially available. Moreover, Rh(acac)(coe)<sub>2</sub> is highly air-sensitive necessitating that the reactions be set up in a nitrogen-filled glovebox,<sup>13</sup> which is inconvenient for most research laboratories.

**Table 1.** Synthesis of various *N*-Boc amines.<sup>13</sup>

| $  \begin{array}{ccc}  \text{BocHN}-\text{CH}(\text{SO}_2\text{Ph})-\text{Ar}^1 & \xrightarrow[\text{K}_2\text{CO}_3 (6 \text{ equiv}), \text{Et}_3\text{N} (1.5 \text{ equiv}), 4\text{\AA} \text{ sieves, dioxane, } 70^\circ\text{C, } 20 \text{ h}]{\begin{array}{c} 5\% \text{ Rh(acac)(coe)}_2 \\ 5.5\% (R,R)\text{-deguPHOS} \\ \text{Ar}^2\text{B(OH)}_2 (2 \text{ equiv}) \end{array}} & \text{BocHN}-\text{CH}(\text{Ar}^2)-\text{Ar}^1  \end{array}  $ |   |   |                        |                     |
|---|---|---|------------------------|---------------------|
| Entry   | <i>Ar</i> <sup>1</sup>                          | <i>Ar</i> <sup>2</sup>                          | Yield (%) <sup>a</sup> | ee (%) <sup>b</sup> |
| 1   | Ph  | 4-ClC <sub>6</sub> H <sub>4</sub>               | 76                     | 98 <sup>c</sup>     |
| 2   | Ph  | 4-MeC <sub>6</sub> H <sub>4</sub>               | 70                     | 96                  |
| 3   | Ph  | 4-MeOC <sub>6</sub> H <sub>4</sub>              | 76                     | 93 <sup>c</sup>     |
| 4   | Ph  | 4-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub> | 51                     | 95 <sup>c</sup>     |
| 5   | Ph  | 3-ClC <sub>6</sub> H <sub>4</sub>               | 55                     | 99                  |
| 6   | Ph  | 3-MeC <sub>6</sub> H <sub>4</sub>               | 66                     | 95                  |
| 7   | Ph  | 3-AcC <sub>6</sub> H <sub>4</sub>               | 52                     | 94                  |
| 8   | Ph  | 2-MeC <sub>6</sub> H <sub>4</sub>               | 62                     | 93                  |
| 9   | 4-MeC <sub>6</sub> H <sub>4</sub>               | Ph  | 71                     | 90                  |
| 10  | 3-MeC <sub>6</sub> H <sub>4</sub>               | Ph  | 70                     | 95                  |
| 11  | 2-MeC <sub>6</sub> H <sub>4</sub>               | Ph  | 63                     | 97                  |
| 12  | 4-BrC <sub>6</sub> H <sub>4</sub>               | Ph  | 59                     | 90                  |
| 13  | 2-thienyl                                       | Ph  | 71                     | 96                  |
| 14  | 4-MeOC <sub>6</sub> H <sub>4</sub>              | Ph  | 76                     | 96 <sup>c</sup>     |
| 15  | 4-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub> | Ph  | 69                     | 79 <sup>c</sup>     |

<sup>a</sup> Isolated yields after chromatography. <sup>b</sup> Determined by chiral HPLC analysis. <sup>c</sup>

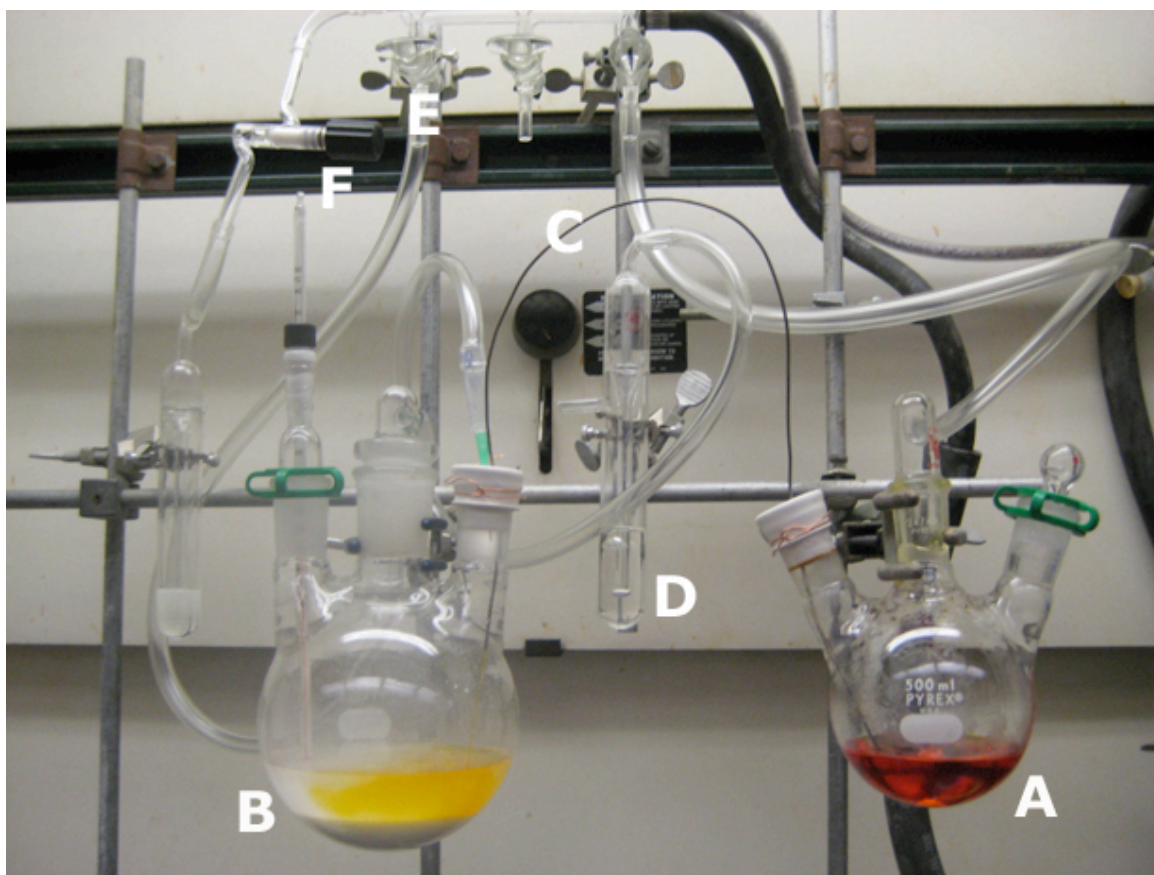
Absolute configuration established by comparison of the optical rotation of amine obtained upon Boc cleavage to literature values.<sup>14</sup>

Optimization of this chemistry was therefore revisited. We found that the inexpensive and air-stable precatalyst, [RhCl(cod)]<sub>2</sub>, performed equally well to Rh(acac)(coe)<sub>2</sub>. Unfortunately, an enantiomeric excess of only 40% was achieved with this precatalyst. To improve the enantiomeric excess, we

therefore performed a series of preincubation experiments whereby  $[\text{RhCl}(\text{cod})]_2$  and (*R,R*)-deguPHOS were stirred at 70 °C in dioxane prior to adding the starting materials, bases and molecular sieves. We found that one hour of preincubation resulted in a dramatic improvement in the enantiomeric excess to at least 95% ee. Shorter preincubations gave lower enantiomeric excess, whereas longer incubations were not beneficial.

Furthermore, we discovered that the presence of significant quantities of the boroxime (cyclic anhydride) in the boronic acid resulted in a decreased yield of the title compound **2**. Decreased yields may occur because the boroxime adds only slowly to the *in situ* generated imine, which competitively hydrolyzes under the reaction conditions. Commercially available boronic acids contain varying amounts of boroxime and therefore should be recrystallized from water before use. To avoid too much water in the reaction mixture, the boronic acid should also be dried prior to use. Boronic acids should not contain more than 5% boroxime and preferentially no more than 30% water as determined by  $^1\text{H}$  NMR in dry  $\text{DMSO}-d_6$ .

**Figure 1:** Cannulation technique



To expand the usability of the chemistry and to make it easier to perform on larger scale the reaction was set up using Schlenk techniques. This reaction set up provides for efficient reactions on both small and large scale, but it is important to transfer the active catalyst solution by cannulation technique to completely avoid exposure to air.

In conclusion, the title product **2** has been prepared in good yield and with high enantioselectivity. We believe that these optimized conditions should be compatible with the same range of different  $\alpha$ -carbamoyl sulfones and arylboronic acids reported previously (Table 1).<sup>13</sup> This method, which utilizes the commercially available (*R,R*)-deguPHOS chiral ligand and the commercially available and air stable [RhCl(cod)]<sub>2</sub> precatalyst, does not require the use of a glovebox and represents a straightforward and general method for the enantioselective synthesis of *N*-protected diaryl methanamines.

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## Appendix

### Chemical Abstracts Nomenclature; (Registry Number)

*tert*-Butyl phenylsulfonyl(thiophen-2-yl)methylcarbamate: Carbamic acid,  
*N*-[(phenylsulfonyl)-2-thienylmethyl]-, 1,1-dimethylethyl ester;  
 (479423-34-2)

*tert*-Butyl carbamate: Carbamic acid, 1,1-dimethylethyl ester; (4248-19-5)

2-Thiophene-carboxaldehyde; (98-03-3)

Benzenesulfinic acid sodium salt; (873-55-2)

[RhCl(cod)]<sub>2</sub>; (12092-47-6)

(*R,R*)-deguPHOS: Pyrrolidine, 3,4-bis(diphenylphosphino)-1-(*phenylmethyl*)-, (3*R*,4*R*)-; (99135-95-2)

4-Chlorophenylboronic acid: Boronic acid, B-(4-chlorophenyl)-; (1679-18-1)



Morten Storgaard was born in Denmark in 1980. He graduated from Technical University of Denmark in 2006 with a M.Sc. degree in chemistry and in 2007 he continued as a Ph.D. student under the supervision of professor David Tanner and Dr. Bernd Peschke from Novo Nordisk. His research has mainly been focusing on palladium catalyzed coupling reactions towards the synthesis of biologically active compounds. In the summer and fall of 2008 he visited the group of Jonathan A. Ellman at University of California at Berkeley and carried out research on the rhodium-catalyzed enantioselective synthesis of amines.



Jason Bexrud received his B.Sc. from Simon Fraser University in 2003. After which, he began doctoral work at the University of British Columbia with Laurel Schafer. His thesis focused on the development of titanium and zirconium-catalyzed hydroamination and C-H functionalization reactions.

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